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BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

MARCH 1940

PRESIDENTIAL ADDRESS*

MALCOLM GOODRIDGE

JUST one year ago, I reviewed the history of The New York Academy of Medicine, and emphasized the fact that throughout its ninety-two years there had been continued progress. I said at that time that we want to be in a position to do even better work than we have in the past, because, as we grow in years, we take on new responsibilities. We cannot remain static; we go either forward or backward.

In some respects, the Academy has made greater contributions during the past year to public welfare, to the welfare of medical education in general, to the welfare of its Fellowship, than ever before in its history.

In other ways, however, this has been a disappointing year. The finances of the Academy continue to be a matter of great concern, and, because of the necessity for balancing the budget, we have been forced to curtail our activities to such an extent that there is a prospect that we shall not be quite so efficient during the current year as we have been in the recent past. This curtailment of activities in every department has been forced upon us, because the requested budget for 1940 so far exceeds the Academy's income. I regret to say that only a small percentage of the Academy Fellowship has given the Fund-Raising

* Read January 4, 1940 at the Annual Meeting of The New York Academy of Medicine.

Committee its enthusiastic support, either through direct financial contributions or by making individual efforts toward increasing Academy endowment.

Your Budget Committee has had a good deal of advice as to how the budget might be balanced. It has even been suggested that one activity might be dropped entirely. I can tell you that, if all three of the Standing Committees of the Academy were abolished, with their paid staffs, the surplus remaining after the budget had been balanced in this manner would be scarcely over five thousand dollars. And then, one day, the question might again be raised, as to whether the Academy was properly an educational institution, or if it was not rather a private institution maintaining a library and section meetings for the benefit principally of its Fellowship. That would render us open to investigation on the part of various government agencies, to show cause why we are not subject to income and social security taxes, state unemployment insurance contributions, and real estate taxes; and, under those circumstances, if such taxes were applied, we would have to close the doors of the Library to all but Fellows, and undertake thorough reorganization of the entire staff of the Academy.

So we find ourselves in a serious position, faced with the necessity to curtail the activities of various Standing Committees of the Academy and the Library, by cutting ruthlessly their requested budgets for 1940, which must entail the loss of competent, well-trained employees of long standing.

I am not in any way exaggerating the dilemma in which we find ourselves. Be assured, we are going to carry on, carry on with all the enthusiasm which the various Standing Committees and their staffs have shown in the past, in spite of these handicaps. We are not planning at the present time to abolish any Standing Committee, and we hope this will never become necessary.

I think I have made it perfectly plain that, if the Academy is to go on with its service to the public, we shall have to have more endowment. During the last two or three years, a very hard-working Fund-Raising Committee has managed to raise funds sufficient to help carry the burden. It has not been possible, however, to add most of the funds thus raised to endowment; they have in large measure been paid out for current expenses. This is a hand-to-mouth existence which cannot be permitted to go on. We shall need additional endowment, if we are to

continue to accept our responsibilities as we have in the past, and if we are to continue to develop our capacity for public service in normal fashion.

In the review of the activities for the past year, there is, I am thankful to say, a brighter side.

Committee on Library

The Library activity has continued at an increased pace during the year. There has been an increase in readers over any other year; there has been such a steady increase in bound and unbound volumes that the stacks are now filled to capacity, and the catalog drawers are almost filled. The need for a new stack-room, costing in the neighborhood of three hundred thousand dollars, grows constantly more acute. Unless such an addition is forthcoming, many valuable reference books must be stored, and hence made unavailable to Fellows and other readers.

Because of the lack of adequate Library staff, some details of cataloging have had to be dropped entirely. There are still groups of books which have been in our Library for a number of years and have not been cataloged.

During the year, donations of books were received from many individuals and organizations.

So that you may have a clear idea of what our financial situation is, as it affects the Library, in respect to income, I would say that we have not been able to come within twenty thousand dollars of meeting the requested budget of the Library Committee.

I think most of us have come to realize that the word, *academy*, in its modern sense, and as applied to the Academy of Medicine, connotes an institution of learning, or, if you prefer, an association of individuals for the cultivation and advancement of science and for the elevation of the standard of medical education. The value of such an institution varies in direct ratio with the size, growth, and working efficiency of its library. The Academy Library is being very materially crippled by lack of funds.

Committee on Public Health Relations

The Committee on Public Health Relations has rendered its usual public service to the health and welfare problems of the community. Perhaps the outstanding accomplishment of the year of this Committee,

with the financial support of the Josiah Macy, Jr., Foundation, was the two-day round table discussion of convalescent care, from its medical and socio-economic aspects.

This Committee has also studied such a variety of questions in the interest of public health as tuberculosis hospitals, one grade of milk, the conference on marriage consultation centers, the control of prostitution, the evaluation of school lunches, open-air classes in schools, health department personnel, physical standards for admission to training courses for police and firemen, the revision of the Physician's Pocket Manual at the request of the Bureau of Census, the health of adolescents.

Advice and assistance have been given in various ways to health, hospital, and municipal civil service commissions, and in relation to health standards for the Board of Education. It has also given much consideration to bills introduced into the Legislature, as affecting public health.

Committee on Medical Education

The Committee on Medical Education sponsored the Twelfth Annual Graduate Fortnight, in October, last year. This Fortnight has become an important feature of medical education in this city. The Graduate Fortnight last year was far and away the most successful of the twelve thus far held under the auspices of this Committee. The attendance was greater, and, in spite of the expense involved in such an important Fortnight, with its accompanying exhibit, the Fortnight finished with an actual positive balance. In other words, for the first time, this Fortnight was not an expense to the Academy; it paid its way, and had something left over.

This Committee was also responsible for the Friday afternoon lectures, lectures on obstetrics, and supervision of Sections, all of which have been maintained at the usual high standard of recent years. The Committee also publishes the Surgical Bulletin, and maintains the Bureau of Clinical Information.

I am happy to report that the subscriptions to the Academy Bulletin received from non-fellows have increased in number, from a little over two hundred to well over three hundred, in the last twelve months.

The very high standard set in recent years by the Subcommittee responsible for the programs of the Stated Meetings has been held.

Committee on Medical Information

The Committee on Medical Information is just a year old, as a Standing Committee of the Academy. It has rendered many important services as a liaison between the Academy and the public. In this department, some five thousand inquiries were handled during the year, twenty-five per cent of them from physicians, and of this twenty-five per cent, forty-three per cent were from Fellows of the Academy of Medicine. Thus, eighty-nine per cent of the total inquiries came from sources outside the Academy Fellowship.

The Committee has arranged and chosen the speakers for the laity lectures, in which the public has shown sufficient interest to fill Hosack Hall to capacity, and often to overflowing. It has also arranged for the delivery of fifty-two Academy radio periods on a coast-to-coast network through WABC. It instituted a study of the broadcasting of medical education by medical organizations, with the aid of a fellowship grant from Rockefeller Foundation. It has rendered services to an imposing list of organizations too numerous to mention.

It has rendered important service to the public, and has entirely justified its elevation to the rank of Standing Committee of the Academy.

* * * * *

The report of the Committee on Activities was accepted by the Council, and most of its recommendations have already been acted upon.

An important change in the By-Laws has been suggested, and has already been approved by the Council. It will be submitted, with other amendments to the By-Laws, to the Academy for approval at a later date. This particular proposal eliminates the Membership class in the Academy, and makes all Members, Fellows; and necessitates the abolition of the Committee on Fellowship.

* * * * *

I have tried to give you a brief outline, so that you may be acquainted with the activities of your Academy. I said a year ago that I hoped we might stir the consciousness of the people as to the significance of the Academy as a public health counselor. I still hold that hope, for the writing on the wall is obvious enough, that if we cannot demonstrate to the satisfaction of people that the Academy is necessary to the public welfare and therefore deserving of financial support, we cannot continue to discharge our full responsibility to the community.

It is my firm conviction that the reason we do not attract more general financial support from outside our walls is that the public remains uninformed as to our objectives and our accomplishments.

I am sure much can be done toward keeping the Academy functioning *pro bono publico,* if all those who derive benefit from the Academy's activities, including the Fellows, will make the effort to impress these facts upon the community's consciousness.

Before I close this annual statement of the affairs of the Academy, I wish to express a word of appreciation to my immediate predecessor in office, a friend of half a century, James Alexander Miller, for his ever-ready willingness to lend me his ear and to give me wise counsel.

It has been for me a very happy year, due in large measure to the enthusiastic coöperation I have received from the entire Academy staff, and to the fine state of morale which exists among them.

Working with the new Director of the Academy has been a source of particular joy to me. In less than a year, he has proved himself in every way entirely worthy to fill the office whose standards were set by the immortal Linsly Williams and continued by John Hartwell. I consider this the greatest tribute I could possibly pay your Director, Herbert Wilcox.

PHYSIOLOGY OF THE TESTES AND THERAPEUTIC APPLICATION OF MALE HORMONE[1]*

CARL R. MOORE

Professor of Zoölogy, The University of Chicago

Two principal functions characterize the testes and these are recognized to be coöperating agencies in insuring effective male participation for the continuance of the race. The primary function of germ cell production is phylogenetically the older and the hormone secreting capacity a gradually developed, or intensified adjunct, that is responsible for promoting the functional coöperation of accessory reproductive organs that have become increasingly complex as evolution has occurred. Of perhaps equal importance for racial existence are the psychobiological phenomena that constitute the sexual drive, or mating instinct. Granted all other phases normal, the absence of a mating tendency in males would preclude racial existence. Aside from some of the primates, especially man, the lack of testis hormone largely eliminates sexual behavior and even in man the hormone plays a decided role, but to what extent is difficult to define.

SPERMATOGENETIC ACTIVITY

Avoiding for the moment considerations of embryonic development, the testicle passes through a period of slow progressive maturing which is usually accelerated just prior to spermatozoan formation. The age of this attainment is variable in different species; in the rat spermatozoa appear about the 40th day after birth whereas in man the comparable period is close to the 15th year. In the vast majority of vertebrates, spermatozoan production is strictly a seasonal event; this periodic phenomenon largely characterizes the lower vertebrates but it holds likewise for the greater number of mammals. In the ground squirrel of the mid-west (Citellus tridecemlineatus) emergence from hibernation in April reveals the testicle in high spermatogenetic activity and quantities

[1] Several of the investigations referred to here have been aided by a grant from the Rockefeller Foundation to The University of Chicago.

* Presented November 2, 1939 at The New York Academy of Medicine in the Twelfth Graduate Fortnight.

of spermatozoa are present. By June or July sperm are few or absent and by September the aspermatic testicle is approximately 1/20th the weight of the breeding testis. During December and January, in hibernation or in the laboratory, testicular activity is renewed. Thus for more than half the entire year the testicle is essentially an inactive organ.

Intermediate types bridge the gap between this very short seasonal type and the continuously active type of testis possessed by the rat, rabbit, guinea pig and man. In this group gametogenetic activity is continuous from its beginning to decline or extinction—a period which is probably no more strictly dated than the occurrence of a subnormal function on the part of a heart, kidney or stomach. The testicles of one man may cease to manufacture spermatozoa at 40 years, in others not until the 50th or 60th year, whereas some men of 80 years or above may continue to produce spermatozoa in large quantities.

The classical investigations of Smith[1,2] and of Smith and Engle[3] approximately a dozen years ago revealed to us clearly, for the first time, the specific dependence of gonadal activity upon the pituitary gland and strikingly demonstrated that the sex glands were not autonomous organs, capable of regulating their own activity, but were really organs whose structural and functional welfare was at the mercy of their humoral environment; this may include such elements as specific nutrients (vitamins, etc.) as well as activating substances or hormones.

Numerous investigations involving all the vertebrate classes have now clearly emphasized the testicular dependence upon pituitary secretions. If the pituitary body is removed through operation, germ cells are not thereafter produced but the introduction of fresh gland tissue or proper extracts into the pituitary-less animal restores the capacity to form germ cells. Administration of pituitary substance, or extracts, as well as activating substances from other sources, to prepuberal animals has in many cases revealed the capacity of the gonad to assume maturity functions long before it normally does.[3] In mammals, in particular, this has exhibited itself as a stimulation of hormone secretion more than of spermatogenetic activity but in lower vertebrates spermatogenesis has been precociously stimulated by treatments with pituitary substance or extracts in amphibia, birds and to some extent in seasonal mammals. Careful studies on the rat[4] and on monkeys[5] have revealed no evidence of hastening the onset of spermatozoan formation by any treatment. The clinical literature has thus far failed to mention precocious spermato-

genetic responses in man.

The demonstration of the prime role of the pituitary in controlling spermatogenetic activity has in a sense shifted the enquiry into basic regulating forces in control of spermatogenesis from the testicle to the pituitary body. In the seasonally active mammal Wells[6] has demonstrated that reproductive inactivity lies not in lack of capacity of the reproductive system to function but is caused by pituitary failure to produce the necessary stimulating force. During the inactive season reproductive stimulants applied artificially for periods of a few weeks will return the testicle and all parts of the system to a breeding state, but the pituitary gland, though a powerful stimulating agent during the sexually active season, reveals only a low stimulating capacity in the sexually inactive period. Contrasting the continuous, and the seasonally active, rodent types it becomes apparent that in the one there is a continuously active pituitary and in the other an intermittent or seasonally active gland.

Enquiry into the factors governing activity of the pituitary, as a solution to seasonal reproduction, provides us with but scanty information. Some element of the environment, or a group of elements, must represent the ruling forces. Rowan[7] clearly demonstrated, and numerous researches have extended the conception, that relative lengths of the light period to darkness play an important part in reproduction in birds and some mammals. Rowan produced a progressively longer day by means of electric lights after sundown, and as the total daylight period began to approach that of the normal spring breeding period his wild captive bird (Junco) showed increased testicular activity and spermatozoan formation during mid-winter when temperatures of −40 degrees F prevailed; control birds without added light showed inactive testes weighing but a small fraction of the weight of testes from light treated birds. Researches of Bissonnette,[8, 9] Benoit[10] and other investigators have extended the findings to several other birds and some mammals. Benoit not only demonstrated the effectiveness of added light to be through the eye but also that light conducted by a quartz rod passing through the orbit to the pituitary body initiated activity in this gland, which activity in turn was the cause of stimulation of development in the reproductive system. The pituitaries from light stimulated ducks implanted into immature female mammals awakened activity in the reproductive system in contrast to ineffectiveness of pituitaries from ani-

mals not exposed to added light. Relative duration of light therefore is one factor in the environment operating to control pituitary activity in some animals but surely other elements are important and some animals may fail to exhibit reproductive responses to added illumination. Wells[11] determined that when ground squirrels were transferred to a constant low temperature in a breeding state, they maintained this active state for periods of a year whereas all field animals had undergone reproductive involution within a period of two months; presumably retention of the breeding state for unusually long periods in the cold was due to retention of activity in the pituitary gland. Other environmental factors such as temperature changes, in contrast to absolute temperature, appear to induce modifications in the reproductive states. On the whole our information with regard to the forces governing pituitary activity is not extensive. The evidence is strong that testicular injuries induced through withholding vitamin-B requirements, or through inanition, represent a fundamental alteration in pituitary activity.[12] Sex gland hormones likewise influence pituitary physiology, and consequent testicular activity, as will be pointed out later.

A recognition of the basic pituitary control over the gonad suggests that diminished or severely curtailed testicular function may be due in large part to defects elsewhere than in the reproductive system.

Spermatogenetic activity of the testis is influenced by other things than pituitary secretion. Radiations of various kinds are known to destroy the germinal elements hence lack of germ cells is to be expected. In mammals possessing a scrotum the failure of testicular descent, or retention of testes in the abdomen, makes it impossible for germ cell formation.[13] The cause of the aspermatic condition has been revealed through many experimental analyses to be the abnormally high temperature of the abdomen. The scrotum is recognized as a local thermoregulator effectively reducing the temperature of the testicle and this function is essential to the production of germ cells.[14] The closure of the excurrent outlet from the testis, by severing or ligating the vas deferens, has been alleged to result in destruction of the germinal tissues and to prohibit further spermatogenetic activity. This contention deserves passing mention, only because of the continuous resurrection of the idea from the literature of ten to thirty-five years ago. More than a hundred years ago the English physician and surgeon, Sir Astley Cooper,[15] proved conclusively on his own dog that closure of the outlet

passages for periods of three to five years, closure being confirmed at autopsy, left the testicle normal in size and actively producing germ cells. Repeatedly during the past twenty years the vas deferens has been closed without abolishing spermatogenesis in several species of birds and at least eight species of mammals, including rats and man; closure of the passages existed for periods of months, and years in the case of man.[16] Separate pieces of the testicle existing as a transplant from one animal to another, hence without ducts, have continued spermatogenetic activity in amphibia, birds and mammals. The rate of new formation of sperm, after vas closure, may be somewhat reduced, and local areas of destruction and resorption may occur temporarily, probably due to excessive pressure from retention, but when resorption relieves the condition, renewed activity follows.[16] We can agree that vas deferens ligation is an effective sterility measure but only by preventing egress of formed cells and not from lack of capacity to produce them.

Testis Hormone

Although it has been known since Biblical times that early loss of the testicles occasions marked differences in man and other mammals it is less than 100 years since Berthold[17] demonstrated this to be due to a humoral mechanism.

When we survey the problem of testis hormone in its entirety we are forced to attribute the chief significance of the secretion to its capacity to condition the organism successfully to carry on its part in reproduction. Nature apparently did not have in mind cosmetic phenomena, a specific sleep producing mechanism, a means of prolonging life, sharpening of wits, nor any specific aid to digestion, when testis hormone was provided for. Successful performance in reproduction involves among other things a control of function of the accessory reproductive organs; this function provides a means of transport for spermatozoa, their introduction into the female, as well as stimulating the desire to mate with receptive females. The condition of the various accessory reproductive organs serves as a means of indicating the hormone state of the animal; the condition of the comb in cocks, the microscopic character of seminal vesicles, prostate glands, vas deferens, Cowper's gland, etc. have all been employed to denote the presence or absence of testis hormone.

It should be recalled that for the majority of vertebrates the animal

is a going concern throughout the year while the reproductive system may be in a functional condition for periods of but two to three months.

In point of contrast we may refer to animals that secrete hormone continuously and to others in which such secretion is limited to a very short period. In the first case the accessory reproductive organs and mating instinct are fully developed throughout the year and the animal functions in reproduction in any month, whereas in the latter type such conditions prevail perhaps for less than two months and the males at other times of the year are in essentially a castrate condition. The various lines of evidence show clearly that the endocrine function, as well as the gametogenetic function, depends upon the pituitary gland.

In constant or seasonal producers of hormone, pituitary removal causes immediate loss of hormone secretion and effectively prevents further secretion as long as there is an absence of a gonadal stimulating substance.

With reference to the period of hormone secretion it has thus far been impossible to set a very definite beginning. The question whether embryonic testes secrete a hormone that is responsible for the development of the Wolffian ducts is a problem receiving much attention at the present time. In my estimation the majority of the evidence inclines to deny it and if this estimate is correct it would place the beginning of testis secretion in postnatal life, the exact time varying with different species. In the rat evidence points to the 35th to 40th day as a period at which hormone secretion is sufficiently under way to attain an effective threshold for seminal vesicles. Similarly, in other mammals various postnatal periods exist subsequent to which definite signs of beginning puberal changes become evident. In man the studies of prostates throughout life by R. A. Moore[18] give indication of definite testis secretion from the 10th to 13th year of life. Androgens have been recovered from the urine of boys at ages from three to five years, with gradually increasing quantities up to fifteen to twenty years[19,20,21] but it is not certainly established that recovery of androgenic substances from the urine of children indicates clearly the effective release in the body of internal secretions from the testis.

Once established, hormone secretion is a continuous process in several species, including man, and intermittent with seasons in the majority of vertebrate species, until the declining period of its production; this latter period is less well circumscribed than its onset. In the rat no one

has yet obtained males so old that age alone has clearly limited this activity. The oldest rats studied by Romeis[22] which were older than three years—a period much beyond the average span of a rat's life—were in possession of accessory organs larger than those from younger vigorous animals. Such conditions of accessory organs are proof of strong testis hormone secretion. Wiesner[23] failed to find any correlation between extreme age and external manifestations of senility and hormone secretion. In man, R. A. Moore[18] found evidence from prostates of the loss of hormone secretion in some cases by the 50th year, but just as good evidence from others of 80 years that testis secretion was in good order. General physiological states throughout life may modify hormone secretion at any period yet to link so-called senility changes with diminution of hormone secretion in any causative manner is to go beyond the known facts.

It is to be emphasized that hormones secreted by the testis are not stored in the body but are rapidly lost through utilization, breakdown or excretion. Mature rats reveal definite cytological changes in the accessory organs within two or three days after testis removal.

Aside from loss of secreting tissue—which can follow diseases, radiations or removals—the greatest known single factor modifying testis hormone secretion is the pituitary gland, and many imposed conditions that result in loss of hormone secretion appear to be indirect effects through pituitary interference. Inanition, or vitamin-B deprivation leads to a loss of testis hormone secretion[12] but they likewise produce relative inactivity in the pituitary.[24,25] In such deficient males hormone secretion is immediately reinitiated, without dietary correction, by such gonadotropic agents as exist in pregnancy urine or the pituitary. In a similar manner seasonally inactive males lose their testis hormone secreting capacity at a time when the pituitary gland shows a diminished capacity to stimulate the reproductive system of immature females. Such males will secrete hormone intensely if only they are treated with a gonadotropic agent.[6] Any condition, therefore, that involves pituitary inactivity will naturally lead to diminution or loss of testis activity.

Hormone secretion can be carried on by testicular tissue located at any point in the body as a viable graft.[17,26] The mere transfer of testis tissue into a new locality, however, does not constitute a viable graft even though firm nodules may be palpable. Unless the tissue remains viable, after such transfer, no physiological effect can be expected ex-

cepting some unknown effect that may possibly come from the autolysis of any tissue, since the amount of hormone carried over by even an entire testicle is too small to be detected by the most sensitive indicators known.[27] Viable grafts cannot be expected unless the transplanted tissue is from the same species[26] and the alleged effectiveness, for long periods, of ape testis implants into man can be discounted.

Cryptorchid or undescended testes do secrete hormone even though gametogenetic activity is entirely absent. Instead of greater hormone secretion by the undescended testicle, as formerly contended, the production is less in testes artificially elevated into the abdomen as well as those occurring normally.[28,29] Whether this is due to an effect of the higher abdominal temperatures, injurious to the gametogenetic function, or to unknown factors, is yet to be determined.

Vasoligation, as a rejuvenation measure, was popular but a short time past, the idea behind it being based upon the supposition that hormone secretion was increased thereby and that secreted hormone served as a rejuvenation instrument. Long preceding the introduction of vasoligation for alleged rejuvenation purposes, subsequent to 1920, it had been shown incontrovertibly that ligation of the excurrent passages from the testes did not cause degeneration of the germinal epithelium or an increase in interstitial tissue. All contentions of an increase in hormone secretion were based upon subjective criteria. The recent investigations of Poynter[30] on the rat have convincingly demonstrated that the operation is without effect on hormone secretion whether the duration of closure has been weeks, or many months, or longer than a year—a period in the rat much beyond one-third of the animal's entire life span.

Isolation of the Male Hormone

A brief recall of the spectacular advances in the last fifteen years in the isolation of the male hormone may be of interest. McGee[31] in 1927 succeeded in preparing the first unquestioned potent extract of the testicle. The lipoid fraction of fresh bull testis provided an impure extract which caused growth of the capon's comb[32] and it proved capable also of repairing castration damage to all accessory reproductive organs of the rat, substituted for the spermatozoon life-maintaining property of testis secretion, and prevented loss (or reëstablished) the power of guinea pigs to perform ejaculation upon electrical stimulation on the head.[16] Further purification of testis extracts by Gallagher and Koch finally

resulted in a highly concentrated activity. In 1929 an androgenic substance was obtained from human urine by Funk and Harrow[33] and by Loewe and Voss[34] which likewise substituted for testis activity. Following the concentration of the active principle in human urine the brilliant chemical work by Butenandt and co-workers[35,36] resulted in obtaining a pure chemical substance, androsterone, whose chemical nature was determined from a very small quantity of crystals. With the proposed formula assigned by Butenandt in mind Ruzicka and co-workers[37] in Switzerland immediately prepared the substance by synthetic means from cholesterol. A second substance having androgenic properties, dehydroandrosterone, was likewise prepared from urine concentrates.[38]

The comparative activities of androgenic substances from urine and from testis tissue on experimental test animals was emphasized especially by the Laqueur group in Amsterdam[39] and differences in the resistance of the active substance from the two sources to boiling alkali[40] indicated that different chemical activities were obtained from the two sources. Further chemical work in Amsterdam finally led to the preparation of a pure chemical substance from testis tissue, testosterone, by David and others.[41] Almost immediately its production by synthetic means was announced by the Butenandt and Ruzicka groups.[42,43] Noting that the effectiveness of testosterone was enhanced by the addition of tissue extracts from testis or other organs, themselves inactive (so-called X-substances), a large number of esters and other compounds of the parent hormone were prepared and proved to possess androgenic activity in varying degrees. Thus, there is available at present more than a score of pure chemical substances that exert androgenic activity when introduced into the organism.[44,45] One of the most active compounds is testosterone propionate, the substance most usually employed for clinical use. It is current belief that the internal secretion naturally supplied by the testicle in life is perhaps quite similar to testosterone, perhaps in some peculiarly effective chemical combination. It must be admitted, however, that the naturally secreted hormone is not known for certainty and neither is it known whether different species produce different chemical substances as their internal secretion from the testis. Physiologically, testosterone, t-propionate, androsterone and other substances maintain the accessory reproductive organs of castrated mammals in a state entirely similar to that in the normal animal though the quantities of the different substances necessary to maintain normality vary to a

great extent; one substance may be more than ten times as effective by weight as another pure substance.

Administration of the chemically pure androgens for the most part has utilized the subcutaneous or intramuscular injection of solutions of the chemical substances in a light oil. Oral effectiveness has been of low order but recent advancements give promise of some improvement in effectiveness when taken by mouth. Suspensions of the compounds in a lanolin base with application as cutaneous ointments is an effective procedure; some compounds thus administered are more effective than subcutaneous injections.[46] Ointments of testosterone, methyl testosterone, and t-propionate are all effective as cutaneous ointments and essentially in the order as given.[47] Deanesly and Parkes[48] demonstrated that implantation of pure crystalline substances beneath the skin provides probably the most efficient method of application. The ideal sought is naturally the slow but continuous release of the active principle into the circulation, and the greater effectiveness of such compounds as t-propionate is believed to reside in the retarded absorption from injection sites.

Number of Hormones

The question of a multiplicity of hormones secreted by the testis has received considerable attention but in my estimation substantial evidence for the production of more than one substance is yet to be presented. In the rat it is fair to state that no known change dependent upon naturally secreted hormone has resisted repair by a pure chemical compound such as t-propionate.

Effects of Administration of Androgenic Substances

The effects of administration of androgenic substances depends to some extent upon the state of the animal receiving treatments. Speaking in general terms for experimental animals: (1) these substances in the developing animal lead to modification of sexual differentiation, particularly in developing females; females of developing chickens,[49,50] rats,[51] mice,[52] and opossums[53,54] are modified in many ways. Complete male duct equipment may be produced in females; copious amounts of prostatic tissue may be caused to develop in females that normally never exhibit the prostatic homologue; and the female external genitalia may be caused to develop into typical masculine type. (2) Administration of androgenic substances to immature males induces precocity that

involves all duct and glandular structures of the reproductive system; in some species the testis appears to exhibit precocious development whereas in other species definite and pronounced injury follows. Precocious puberty, a term applied sometimes unfortunately, will perhaps convey the general effects here indicated. Chicks out of the egg only two weeks respond to male hormones by lusty crowing and attempts at treading their immature mates whether the substances are secreted by the testes as a result of gonadotropic treatment[55,56] or injected direct.[57] (3) Administration to males below par in their sexual states can result in greatly stimulated responses.[58,59,60] Extra-seasonal males can be returned to the breeding state in short order. Low copulatory responses can be replaced by decided activity. Underdeveloped accessory organs of reproduction—prostates, seminal vesicles, penis, etc.—are increased in size. (4) Administration to adult castrated males prevents regression of the accessory organs if it follows castration immediately, or repairs the involuted castrate organs that are dependent upon such secretions. These hormones enhance, or maintain, or stimulate, as the case may be, the sexual drive. (5) Administration of androgenic substances to females has a variety of effects. In the normal female estrus cycles and ovulation are abolished; ovaries are frequently luteinized; menstruation is inhibited. Male psychic tendencies are frequently induced and female rats adopt the masculine sexual responses; female canary birds are induced to sing and their social order is materially advanced; courtship is of a masculine type.[61] Mammary glands and uterus are often stimulated. During pregnancy androgenic treatment may be followed by death, resorption or sexual modification of young; parturition is postponed and lactation is essentially abolished.

Various other additional effects could be mentioned but it is sufficient, perhaps, to realize that though these substances play an important role in normal masculine sex life, untoward effects may follow their application as an ordinary drug.

Of particular interest here, are the effects of androgenic substances in man. Of what use are they clinically; in what type of patient should they be employed; what is proper dosage; how administered; what contra-indications exist? The general statement that the clinical limitations are not yet completely defined can pave the way for certain suggestions arising from hormone applications in man, guided often by our knowledge of effects in experimental animals.

It will no doubt suggest itself immediately that individuals devoid of testicular tissue through lack of proper development, atrophy or castration would be proper subjects for therapy. By such means lack of maturity changes associated principally with a high pitched voice may be expected to show modifications. Alleviation of castration or eunuchoidal conditions reported by clinicians, usually after subcutaneous or intramuscular injections, have largely followed a general sequence in which earliest noticeable changes have been the initiation of, or more frequent, erections sometimes characterized by severe priapism; growth of the penis; induction of, or increase in, ejaculations; growth of genital hair, prostate; increased salt, water and nitrogen retention with resulting gain in weight; some change in pitch of voice and less frequently, perhaps, growth of beard. After the principal puberal changes have been developed, retrogressive changes of more evident nature do not appear rapidly with discontinuance of hormones, but involution of accessory reproductive organs does occur. It is to be appreciated, however, that the maintenance of a typical functional state is dependent upon continuous administration. Daily dosages of 25 mgs. t-propionate have been found effective, as well as dosages divided to approximate 50 to 60 mgs. per week.[62 to 68] Analysis of the urine of eunuchoids or castrates reveals an increase in excreted androgens during administration and the recovery of activity has been reported from 14 per cent to 70 per cent, the variations being due in part to an estimate based upon different urinary compounds.[69,70,71,72]

The question of effects of administered androgens upon the testicle is not settled. Against the supposition that testis hormone would stimulate testicular activity Moore and Price have emphasized the injurious effects upon the normal testicle from administration of these hormones to normal young rats.[73,74,75] Short periods of treatment reduced the weight of testicles by 80 per cent, and caused severe histological damage and an entire cessation of spermatozoon production. It was suggested that androgens stimulated the accessory reproductive organs direct but that inhibitory effects of the hormone upon the pituitary was the pathway of the harmful effects of androgens upon the testicle, hence an indirect one. The report of lack of reparative effects upon testicles degenerate because of hypophysectomy, or other causes of damage, while the accessory organs were stimulated was one line of evidence suggesting that androgens had no direct action upon the testicle. These

observations have been substantiated but the fact that androgen administration immediately following hypophysectomy, before damage appeared in the testicle, prevents testicular involution that would otherwise follow has reintroduced the question of testicular stimulation by androgens.[76,77,78] There is the added fact that androgenic substances administered to the quiescent seasonal breeding male cause resumption of testicular activity.[79] These phenomena require further analysis. The fact that hypophysectomy damage is equally well prevented by yeast extracts[80] and by progesterone,[81] and that the protective action of various androgens does not parallel their androgenic potency[78] raises seriously the question of the validity of the evidence suggesting a stimulating influence arising from the testicle upon its own physiological functions.

In addition to the harmful effects upon the testicle of the rat similar injuries have likewise been reported after androgenic treatments in dogs,[82] ducks,[83] cocks[84] and guinea pigs.[85] In man the effects of administered androgens upon the testicle are reported as suggestive stimulation, no apparent effect, or injurious effects. Heckel[86] has recently pointed out severe reduction in spermatozoa in man during injection of t-propionate, with recovery after lapse of administration, and this receives confirmation in the work of McCullagh;[87] repeated periods of injection are followed by severe reduction in sperm counts.

Androgens have been administered to boys in attempting correction of testicular undescent. Whereas some favorable effects are reported the question has other aspects. Precocious development of external genitalia and development of other maturity phenomena introduce questions of the advisability of such ministrations.[88,89]

The treatment of prostatic hypertrophy through administration of androgens has been recommended but opinions are variable on the advisability of the procedure or benefits derived. The treatment has been advocated on the assumption that older men secrete less androgenic materials and that the disturbed normal balance of androgen-estrogen permits estrogenic substances to stimulate prostatic hypertrophy. Suggestions for its use arose largely from the work of the Laqueur group[90] and the clinical experiences of van Cappellen.[91] Zuckerman[92] has discussed the experimental basis suggesting the treatment and the recent literature review of Vidgoff[93] portrays the experiences of a number of workers. Thus far the chief claims for benefit from the treatment rest almost solely upon symptomatology, or clinical improvement,[94] and no

one to my knowledge has claimed reduction in size of the prostate. It is reported that administration of t-propionate for periods up to 95 days fails to alter the histological character of the benign hypertrophied tissues.[95] It would seem fair to suggest that in man prostatic hypertrophy has not been shown to be accompanied by increased urinary estrogens, or the administration of androgens to lower estrogenic output; it has not been shown that estrogens in man are in any way causally related to prostatic hypertrophy; finally, it is scarcely probable, on theoretical grounds, that androgen administration would in any way reduce the size of the prostate. The value of the treatment would appear to be an unsettled question perhaps with the weight of evidence pointing to the negative.

In a discussion of the therapeutic application of male hormone brief mention should probably be made of its use in gynecological practice. Some clinicians have considered it advisable to attempt modifications in pituitary activity by means that would avoid the effects of administrations of estrogens, hence androgens have been tried in various disorders in women. Favorable results have been reported in the inhibition of lactation during the puerperium,[96] amelioration of dysfunctional uterine bleeding,[97,98,99,100] and in menopausal disturbances.[101] Prolonged usage however may be responsible for the induction of undesirable characters such as voice changes, unusual growth of hair or excessive growth of the clitoris.[102]

It is apparent that the general subject of therapeutic administration of the male sex hormone is still in the experimental stage and requires the maturing influence of time and experience before its real value and limitations are established.

REFERENCES

1. Smith, P. E. The disabilities caused by hypophysectomy and their repair, *J.A.M.A.*, 1927, *88:* 158.
2. Smith, P. E. Hypophysectomy and a replacement therapy in the rat, *Am. J. Anat.*, 1930, *45:* 205.
3. Smith, P. E. and Engle, E. T. Experimental evidence regarding the rôle of the anterior pituitary in the development and regulation of the genital system, *Am. J. Anat.*, 1927, *40:* 159.
4. Moore, C. R. Responses of immature rat testes to gonadotropic agents, *Am. J. Anat.*, 1936, *59:* 63.
5. Engle, E. T. Experimentally induced descent of the testis in the Macacus monkey by hormones from the anterior pituitary and pregnancy urine, *Endocrinology*, 1932, *16:* 513.
6. Wells, L. J. Seasonal sexual rhythm and its experimental modification in the male of the thirteen-lined ground squirrel (Citellus tridecemlineatus), *Anat. Rec.*, 1935, *62:* 409.

7. Rowan, W. Experiments in bird migration; manipulation of the reproductive cycle; seasonal histological changes in the gonads, *Proc. Boston Soc. Nat. Hist.*, 1929, *39:* 151.
8. Bissonnette, T. H. Experimental modification of the sexual cycle in males of the European starling (Sturnus vulgaris) by changes in the daily period of illumination and of muscular work, *J. Exper. Zool.*, 1931, *58:* 281.
9. Bissonnette, T. H. Sexual photoperiodicity, *J. Heredity*, 1936, *27:* 170.
10. Benoit, J. Facteurs externes et internes de l'activité sexuelle; stimulation par la lumière de l'activité sexuelle chez le canard et la cane domestiques, *Bull. biol. d. l. France et d. l. Belgique*, 1936, *70:* 487.
11. Wells, L. J. Prolongation of breeding capacity in males of an annual breeding rodent (Citellus tridecemlineatus) by constant low temperature, *Anat. Rec.*, 1935, *64*, suppl. 1: 138.
12. Moore, C. R. and Samuels, L. T. The action of testis hormone in correcting changes induced in the rat prostate and seminal vesicles by vitamine B deficiency or partial inanition, *Am. J. Physiol.*, 1931, *96:* 278.
13. Moore, C. R. Testicular reactions in experimental cryptorchidism, *Am. J. Anat.*, 1924-25, *34:* 269.
14. Moore, C. R. The biology of the mammalian testis and scrotum, *Quart. Rev. Biol.*, 1926, *1:* 4.
15. Cooper, A. P. *Observations on the structure and diseases of the testis.* London, Longman, 1830.
16. Moore, C. R. Biology of the testes, in *Sex and internal secretions.* Baltimore, Williams & Wilkins, 2. ed., 1939, chapt. 7.
17. Berthold, A. A. Transplantation der Hoden, *Arch. f. Anat. u. Physiol. u. Wissensch.*, 1849: 42.
18. Moore, R. A. The evolution and involution of the prostate gland, *Am. J. Path.*, 1936, *12:* 599.
19. Dorfman, R. I., Greulich, W. W. and Solomon, C. I. The excretion of androgenic and estrogenic substances in the urine of children, *Endocrinology*, 1937, *21:* 741.
20. Oesting, R. B. and Webster, B. The sex hormone excretion of children, *Endocrinology*, 1938, *22:* 307.
21. Nathanson, I. T., Towne, L. E. and Aub, J. C. The daily excretion of urinary androgens in normal children, *Endocrinology*, 1939, *24:* 335.
22. Romeis, B. Altern und Verjungung, in *Handbuch der inneren Sekretion* (Hirsch). Leipzig, Kabitzsch, 1933, v. *2*, p. 1745.
23. Wiesner, B. P. Experimental study of senescence, *Brit. M. J.*, 1932, *2:* 585.
24. Evans, H. M. and Simpson, M. E. Subnormal sex-hormone content of the hypophysis of animals with inadequate antineuritic vitamine B, *Anat. Rec.*, 1930, *45:* 216.
25. Mason, K. E. and Wolfe, J. M. The physiological activity of the hypophysis of rats under various experimental conditions, *Anat. Rec.*, 1930, *45:* 232.
26. Browman, L. G. Testicular heterotransplantation in rats and mice, *J. Exper. Zool.*, 1937, *75:* 283.
27. Moore, C. R. The physiological effects of non-living testis grafts, *J.A.M.A.*, 1930, *94:* 1912.
28. Nelson, W. O. Effect of gonadotropic hormone injections upon hypophyses and sex-accessories of experimental cryptorchid rats, *Proc. Soc. Exper. Biol. & Med.*, 1933-34, *31:* 1192.
29. Hanes, F. M. and Hooker, C. W. Hormone production in the undescended testis, *Proc. Soc. Exper. Biol. & Med.*, 1936-37, *35:* 549.
30. Poynter, H. Testis hormone secretion in the rat under conditions of vasectomy or isolation, *Anat. Rec.*, 1939, *74:* 355.
31. McGee, L. C. The effect of the injection of a lipoid fraction of bull testicle in capons, *Proc. Inst. Med.*, 1927, *6:* 242.
32. McGee, L. C., Juhn, M. and Domm, L. V. The development of secondary sex characters in capons by injections of extracts of bull testes, *Am. J. Physiol.*, 1928, *87:* 406.
33. Funk, C. and Harrow, B. The male hormone, *Proc. Soc. Exper. Biol. & Med.*, 1929, *26:* 325; 569.

34. Loewe, S. and Voss, H. E. Gewinnung, Eigenschaften und Testierung eines männlichen Sexualhormons, Akad. d. Wissenschaften in Wien, *Akad. Anzeiger*, 1939, No. *20*.
35. Butenandt, A. Über die chemische Untersuchung der Sexualhormone, *Ztschr. f. ang. Chem.*, 1931, *44:* 905.
36. Butenandt, A. and Tscherning, K. Über Androsteron; ein krystallisiertes männliches Sexualhormon, *Ztschr. f. physiol. Chem.*, 1934, *229:* 167.
37. Ruzicka, L. *et al.* Über die Synthese des Testikelhormons (Androsteron) und Stereoisomerer desselben durch Abbau hydrierter Sterine, *Helv. chim. Acta*, 1934, *17:* 1395.
38. Butenandt, A. and Dannenbaum, H. Über Androsteron; Isolierung eines neuen physiologisch unwirksamen Sterin-derivates aus Männerharn, seine Verknupfung mit Dehydro-androsteron und Androsteron, *Ztschr. f. physiol. Chem.*, 1934, *229:* 192.
39. Laqueur, E., David, K., Dingemanse, E. and Freud, J. Über männliches Hormon; Unterschied von Androsteron aus Harn und Testosteron aus Testis, *Acta brev. Neerland.*, 1935, *5:* 84.
40. Gallagher, T. F. and Koch, F. C. The effect of alkali on the testicular hormone, *J. Biol. Chem.*, 1934, *104:* 611.
41. David, K. *et al.* Über krystallinisches männliches Hormon aus Hoden (Testosteron), wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron, *Ztschr. f. physiol. Chem.*, 1935, *233:* 281.
42. Butenandt, A. and Hanisch, G. Über Testosteron; Umwandlung des Dehydro-androsterons in Androstendiol und Testosteron, *Ztschr. f. physiol. Chem.*, 1935, *237:* 89.
43. Ruzicka, L. and Wettstein, A. Über die künstliche Herstellung des Testikelhormons Testosteron, *Helv. chim. Acta*, 1935, *18:* 1264.
44. Koch, F. C. The biochemistry of androgens, in *Sex and internal secretions.* Baltimore, Williams & Wilkins, 2. ed., 1939, chapt. 12.
45. Gustavson, R. G. The bioassay of androgens and estrogens, in *Sex and internal secretions.* Baltimore, Williams & Wilkins, 2. ed., 1939, chapt. 14.
46. Moore, C. R., Lamar, J. K. and Beck, N. Cutaneous absorption of sex hormones, *J.A.M.A.*, 1938, *111:* 11.
47. Scott, B. L. *Unpublished.*
48. Deanesly, R. and Parkes, A. S. Factors influencing the effectiveness of administered hormones, *Proc. Roy. Soc. London*, ser. B, 1937, *124:* 279.
49. Wolff, E. Sur l'action de l'hormone male (androstérone) injectée à l'embryon de poulet, *Compt. rend. Soc. de biol.*, 1935, *120:* 1312.
50. Willier, B. H., Gallagher, T. F. and Koch, F. C. The modification of sex development in the chick embryo by male and female sex hormones, *Physiol. Zool.*, 1937, *10:* 101.
51. Greene, R. R., Burrill, M. W. and Ivy, A. C. The experimental production of intersexuality in the female rat, *Am. J. Obst. & Gynec.*, 1938, *36:* 1038.
52. Raynaud, A. Intersexualité obtenue expérimentalement chez la souris femelle par action hormonale, *Bull. biol. d. l. France et d. l. Belgique*, 1938, *72:* 297.
53. Moore, C. R. Modification of sexual development in the opossum by sex hormones, *Proc. Soc. Exper. Biol. & Med.*, 1939, *40:* 544.
54. Burns, R. K. The effects of crystalline sex hormones upon sex differentiation in the pouch young of the opossum, *Anat. Rec.*, 1939, *73*, suppl. 2: 61.
55. Domm, L. V. Precocious development of sexual characters in the fowl by homeoplastic hypophyseal implants; the male, *Proc. Soc. Exper. Biol. & Med.*, 1931-32, *29:* 308.
56. Domm, L. V. and Van Dyke, H. B. Precocious development of sexual characters in the fowl by daily injections of hebin, *Proc. Soc. Exper. Biol. & Med.*, 1932-33, *30:* 349.
57. Hamilton, J. B. Precocious masculine behavior following administration of synthetic male hormone substance, *Endocrinology*, 1938, *23:* 53.
58. Stone, C. P. Sex drive, in *Sex and internal secretions.* Baltimore, Williams & Wilkins, 2. ed., 1939, Chapt. 23.
59. Stone, C. P. Copulatory activity in adult male rats following castration and injections of testosterone propionate, *Endocrinology*, 1939, *24:* 165.

60. Stone, C. P. Activation of impotent male rats by injections of testosterone propionate, *J. Comp. Psychol.*, 1938, *25:* 445.
61. Shoemaker, H. H. Effect of testosterone propionate on behavior of the female canary, *Proc. Soc. Exper. Biol. & Med.*, 1939, *41:* 299.
62. McCullagh, E. P., McCullagh, D. R. and Hicken, N. F. Diagnosis and treatment of hypogonadism in the male, *Endocrinology*, 1933, *17:* 49.
63. Hamilton, J. B. Treatment of sexual underdevelopment with synthetic male hormone substance, *Endocrinology*, 1937, *21:* 649.
64. Foss, G. L. Effect of testosterone propionate on a post-puberal eunuch, *Lancet*, 1937, *2:* 1307.
65. Vest, S. A. and Howard, J. E. Clinical experiments with the use of male sex hormones; use of testosterone propionate in hypogonadism, *J. Urol.*, 1938, *40:* 154.
66. Kenyon, A. T. The effect of testosterone propionate on the genitalia, prostate, secondary sex characters and body weight in eunuchoidism, *Endocrinology*, 1938, *23:* 121.
67. Kenyon, A. T. *et al.* The effect of testosterone propionate on nitrogen, electrolyte, water and energy metabolism in eunuchoidism, *Endocrinology*, 1938, *23:* 135.
68. Turner, H. H. The clinical use of synthetic male sex hormone, *Endocrinology*, 1939, *24:* 763.
69. Dorfman, R. I. and Hamilton, J. B. Urinary excretion of androgenic substances after intramuscular and oral administration of testosterone propionate, *J. Clin. Investigation*, 1939, *18:* 67.
70. Callow, N. H., Callow, R. K. and Emmens, C. W. The effect of the administration of testosterone propionate on the urinary excretion of compounds allied to the steroid hormones, *J. Endocrinol.*, 1939, *1:* 99.
71. McCullagh, E. P., Rumsey, J. M. and Cuyler, W. K. Excretion of urinary androgens following the injection of testosterone propionate, *Endocrinology*, 1939, *24:* 833.
72. Hoskins, W. H. *et al.* The effect of testosterone propionate on the urinary excretion of androgens and estrogens in eunuchoidism, *Endocrinology*, 1939, *24:* 702.
73. Moore, C. R. and Price, D. Gonad hormone functions and the reciprocal influence between gonads and hypophysis, with its bearing on the problem of sex hormone antagonism, *Am. J. Anat.*, 1932, *50:* 13.
74. Moore, C. R. and Price, D. Some effects of synthetically prepared male hormone (androsterone) in the rat, *Endocrinology*, 1937, *21:* 313.
75. Moore, C. R. and Price, D. Some effects of testosterone and testosterone-propionate in the rat, *Anat. Rec.*, 1938, *71:* 59.
76. Walsh, E. L., Cuyler, W. K. and McCullagh, D. R. The physiologic maintainance of the male sex glands, *Am. J. Physiol.*, 1934, *107:* 508.
77. Nelson, W. O. and Gallagher, T. F. Some effects of androgenic substances in the rat, *Science*, 1936, *84:* 230.
78. Nelson, W. O. Some factors involved in the control of the gametogenic and endocrine functions of the testis, *Cold Spring Harbor Symposia on Quantitative Biology*, 1937, *5:* 123.
79. Wells, L. J. and Moore, C. R. Hormonal stimulation of spermatogenesis in the testis of the ground squirrel, *Anat. Rec.*, 1936, *66:* 181.
80. Hisaw, F. L., Greep, R. O. and Fevold, H. L. Pituitary-like effects of yeast extracts, *Anat. Rec.*, 1936-37, *67*, suppl. 1: 50.
81. Nelson, W. O. The effect of various sex hormones on the testes of hypophysectomized rats, *Anat. Rec.*, 1936-37, *67*, suppl. 1: 110.
82. Itho, M. and Kon, T. Action de l'hormone male sur les organes génitaux de jeunes chiens males, *Compt. rend. Soc. de biol.*, 1935, *120:* 678.
83. Bulliard, H. and Ravina, A. Effets de la testostérone chez Cairina, *Compt. rend. Soc. de biol.*, 1938, *127:* 525.
84. Morató-Manaro, J. and Albrieux, A. The effect of sex hormones on the combs of castrated and normal cocks, *Endocrinology*, 1939, *24:* 518.

85. Bottomley, A. C. and Folley, S. J. The effect of high doses of androgenic substances on the weight of the testes, accessory reproductive organs and endocrine glands of young male guinea-pigs, *J. Physiol.*, 1938-39, *94:* 26.
86. Heckel, N. J. Production of oligospermia in a man by the use of testosterone propionate, *Proc. Soc. Exper. Biol. & Med.*, 1939, *40:* 658.
87. McCullagh, E. P. and McGurl, F. J. The clinical use of testosterone propionate, *J. Urol.*, 1939, *42:* 1265.
88. Kunstadter, R. H. The induction of premature puberty with androgenic substance, *Endocrinology*, 1938, *23:* 661.
89. Thompson, W. O. and Heckel, N. J. Undescended testes; present status of glandular treatment, *J.A.M.A.*, 1939, *112:* 397.
90. Laqueur, E. Behandlung der Prostatahypertrophie mit männlichem Hormon (Hombreol) und experimentelle Begründung dieser Therapie, *Schweiz. med. Wchnschr.*, 1934, *64:* 1116.
91. van Cappellen, D. Versuch einer Therapie mit Sexualhormon im besonderen männlichen Hormon (Hombreol) bei Prostatahypertrophie, *Deutsche med. Wchnschr.*, 1933, *59:* 726.
92. Zuckerman, S. The endocrine control of the prostate, *Proc. Roy. Soc. Med.* 1936, *29:* 1557.
93. Vidgoff, B. The hormonal control of the prostate and its relation to clinical prostatic hypertrophy, *J. Urol.*, 1939, *42:* 359.
94. Cary, F. S. Synthetic male hormone in the treatment of prostatic hypertrophy, *Illinois M. J.*, 1938, *73:* 486.
95. Moore, R. A. and McLellan, A. M. A histological study of the effect of the sex hormones on the human prostate, *J. Urol.*, 1938, *40:* 641.
96. Kurzrok, R. and O'Connell, C. P. The inhibition of lactation during the puerperium by testosterone propionate, *Endocrinology*, 1938, *23:* 476.
97. Geist, S. H., Salmon, U. J. and Gaines, J. A. The use of testosterone propionate in functional bleeding, *Endocrinology*, 1938, *23:* 784.
98. Hamblen, E. C. Therapeutic use of the sex sterols in functional meno-metrorrhagia, *Endocrinology*, 1939, *24:* 13.
99. Papanicolaou, G. N., Ripley, H. S. and Shorr, E. Suppressive action of testosterone propionate on menstruation and its effects on vaginal smears, *Endocrinology*, 1939, *24:* 339.
100. Mazer, C. and Mazer, M. The treatment of dysfunctional bleeding with testosterone propionate, *Endocrinology*, 1939, *24:* 599.
101. Kurzrok, L., Birnberg, C. H. and Livingston, S. H. The treatment of the female menopause with male sex hormone, *Endocrinology*, 1939, *24:* 347.
102. Greenhill, J. P. and Freed, S. C. Virilism in women caused by androgenic therapy for menstrual disturbances, *J.A.M.A.*, 1939, *112:* 1573.

THE PHYSIOLOGY OF THE OVARIES*

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IT IS WELL recognized that the ovaries have a double function, the formation and periodic liberation of sex cells and the secretion of the female sex hormones, the estrogens and progesterone, which play an important role in the maintenance and cyclical changes of the accessory reproductive organs. It is also clear that the ovaries are not self-maintaining structures. Certain hormones of the anterior pituitary are essential to their maintenance and in turn the secretions of the ovaries influence the secretion of the gonadotropic hormones and probably other hormones of the hypophysis.

The inter-relations of these various structures have been intensively studied in the clinic and experimental laboratory for more than a decade. Although they appeared to be simple at first and in their basic outlines are simple, yet they have many complex features which even at the present time are but imperfectly known. Evidence for this last statement can be gained by even casual examination of results obtained in any sterility clinic, or of experimental work directed towards the reinstitution of fertility when sterility has been induced by the experimental ablation of the hypophysis. We also have, perhaps, been too inclined to believe that the ovarian hormones act solely on the accessory reproductive organs, whereas in fact these secretions are powerful agents which certainly influence many other body mechanisms, perhaps all of them.

The developmental and later cyclical changes of the structural components of the ovary need, as an introduction, to be only hastily reviewed. At birth an immense number of ova are present. Figures differ somewhat but show that there are between 100,000 and 400,000 in the human ovary at birth. Between birth and puberty many of these disappear but there still remains a large number at the onset of sexual maturity (Schroeder[1]). In addition to this initial number, most recent investiga-

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tions indicate that there is a formation of new ova from the germinal epithelium throughout the reproductive period (Allen,[2] Evans and Swezy[3] and others), although this is not universally accepted as being true (Kingsbury[4]). In the human, all ova are destined to mature or to undergo degeneration and destruction by or shortly after the end of the reproductive period, for within a short time after the menopause none can be found nor can follicular development be stimulated by the injection of gonadotropic hormone. It is evident that but few of the ova and their enclosing follicles have undergone complete development, the number of eggs that normally mature during the entire reproductive period of a woman not exceeding some 400. Thus most of the original thousands of primordial follicles degenerate at some stage in their life cycle, not infrequently after they have undergone considerable growth or indeed after they have reached nearly mature size. In most mammals the theca interna of those follicles which undergo atresia form cords or masses of epithelioid-like cells, the so-called interstitial tissue of the ovary. In rabbits in which this type of tissue reaches the greatest development, the ovaries are laden with these cells and they were designated as the puberty gland by Ancel and Bouin. That these cells can excrete estrogenic hormone in lower forms is evident from work on hypophysectomized rats. In old-world monkeys and humans, however, interstitial tissue is very scanty or absent and it is difficult to attach any secretory role to it. The hyalinized bodies which form from atresia of follicles in the human can hardly be suspected of having a secretory function.

The changes and differentiation of various parts of those follicles which are destined to undergo maturation and ovulation are well known, and will not be reviewed. It will suffice to refer only to the fact that during this growth two structures are differentiated which, in addition to the ovum, could, from their structure, be expected to form secretions. These are the epithelial lining of the follicle, the granulosa, and the inner layer of the enveloping connective tissue capsule, the theca interna. The cells composing the latter enlarge and assume epithelioid characteristics during the growth of the follicle. It has been thought that the follicular fluid which, as first shown by Allen and Doisy et al[5] in 1924, contains estrogenic hormone, was probably formed by the granulosa. Corner,[6] however, has recently reviewed the evidence in regard to which cells form estrogen and has come to the conclusion that the connective tissue element, the theca interna, and not the granulosa, is the site of

ovarian secretion of estrogenic hormone.

Following rupture of the follicle and extrusion of the ovum at ovulation profound changes take place in the follicle, resulting in the formation of a new structure, the corpus luteum. This newly formed structure secretes a second sex hormone, progesterone. Although a subject of debate at first, it now seems certain that corpora lutea also secrete estrogen.

In addition to the secretion of estrogen and progesterone, there is secreted by the human female a large amount of androgen, as shown by the urinary assays from the laboratories of Koch,[7] of Laqueur[8] and of Callow.[9] That the ovaries may be the site of secretion of a part of this hormone appears probable. The experimental work of Hill[10] in mice and of Deanesly[11] in rats has shown that ovarian grafts made into the ears of castrated males maintained the male accessory reproductive organs if the animals were placed in a cool environmental temperature. Due to their exposed position, the temperature of the transplanted ovaries was more influenced than they would be in their normal location. When the animals were kept in a warm room the accessory organs atrophied and assumed the condition characteristic of the castrated male, indicating that no androgen was secreted by the ovarian grafts. However, the evidence indicates that in women the most important site of formation of androgen is not the ovaries, but is probably the adrenal cortex. Hamblen et al,[12] using the colorimetric method of Oesting, found normal androgenic titers in five oöphorectomized women and Hirschmann[13] chemically isolated dehydroandrosterone and androsterone from nine ovariectomized women in only slightly lower than the yields reported by Callow and Callow.[14]

Although estrogen is found in the blood and urine in ovariectomized women, as shown by Frank et al,[15] Fluhmann[16] and others, and estrone and progesterone normally occur in small amounts in the adrenals, it seems safe to assert that the effective estrogen and progesterone in nonpregnant women are formed in the ovaries, as evidenced by the profound involution of the accessory reproductive organs after ovariectomy. Where the estrogen which is found in the blood and urine after ovariectomy comes from is an unsolved problem, but the adrenals are a likely source.

Before taking up the physiological actions of the female sex hormones —the estrogens and progesterone—I will discuss in as simple a way as

possible the number of natural estrogens which occur, their structural formulae and the transformations which they undergo before being excreted in the body, or in other words, their metabolism. It is known that but little of an injected estrogen can be recovered from the urine and feces. Furthermore, much that can be recovered is in an inactive form. It has been conjugated and in order to render it again biologically active it must be hydrolyzed. The site in the body where this inactivation or detoxification takes place will also be discussed. I do not find in the literature a simplified description of the chemistry of the estrogens and it seems that an attempt to give such a description might be of value to those who are interested in the sex hormones but who, like the speaker, are not chemists.*

A very large number—running into the hundreds—of estrogenic substances are now known. Their structural formulae have been determined and they have been synthesized in the laboratory. They can be separated into those which occur in nature, or the natural estrogens, and those which do not occur naturally, but which are produced only in the chemical laboratory. Most of the latter are of low potency, although some give a reaction with small amounts of material, as, for example, stilboestrol. Only seven estrogens are known to occur in the tissues and fluids of animals. Of these seven, five are not found in the urine of women but occur in the urine of pregnant mares. Three only (estrone, estriol and estradiol) are found in the urine of women. The third one named—estradiol—has only recently been isolated from the urine of women and occurs in very small amounts. It has also been isolated from the urine of pregnant mares and, more important from the point of view of the metabolism of the estrogens, from the liquor folliculi of sow ovaries, Doisy and his group[17] in 1935 having isolated 11 mg. using more than four tons of sow ovaries to secure this small amount of chemically pure hormone. Since this substance is the one chiefly found in the ovaries, it is thought to be the parent hormone of estrone and estriol, the two other hormones isolated in humans. The naturally occurring estrogens all have the same carbon skeleton, the cyclopentenophenanthrene nucleus (Figure 1). This same skeleton is found in other biologically active substances, bile acids, toad-poisons, androgens, adrenal cortex hormone, progesterone, and the sterols. The differences between these substances

* Invaluable assistance was received from Dr. Louis Levin in the preparation of the discussion on the chemistry of the estrogens and progesterone.

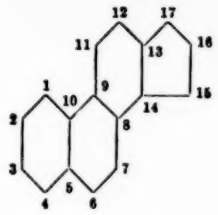


Fig. 1—The carbon skeleton (cyclopenophrenanthrene nucleus) of the naturally occurring estrogens and other steroid hormones, showing the numbering of the carbon atoms.

are due to different side chains and groupings attached to a common skeleton, and to certain space relationships. However, the difficulty of synthesizing the estrogens is much greater than it is with the androgens, progesterone and desoxycorticosterone. The latter are prepared commercially from the natural sterols such as cholesterol. The so-called pseudosynthesis of estrone from ergosterol has been reported but because of the cost of ergosterol and the small yield, it is not a practical method of obtaining estrone. The total synthesis of equilenine, an estrogen found in the urine of pregnant mares, has recently been reported by Bachmann, Cole and Wilds,[18] and since equilenine can be reduced to estradiol the commercial synthesis of this substance appears to be a possibility. All the natural estrogens now in use come from urine, largely from the urine of pregnant mares. This urine yields largely estrone and this by reduction is changed to the more potent compound, estradiol.* Other substances may be substituted in one of the side chains, to give a more prolonged action, as for instance, estradiol benzoate (the commercial product called Progynon B), or estradiol dipropionate (Figure 2).

Chemically the three estrogens differ from each other only in the groupings on carbon 16 and 17 (Figure 2). Estradiol, the most active and probably the true follicular hormone, has an alcohol group on carbon 17. In estrone this alcohol group is replaced by a carbonyl (ketone) group. If the elements of water are added to estrone at the carbonyl group, estriol results. It is evident, then, that these three compounds are very closely related. In fact, some of the interconversions

* Estradiol occurs in two forms, α-estradiol and β-estradiol, the differences being due to the space relationships of the OH group on carbon 17. The α form predominates in the isomers obtained from the reduction of estrone to estradiol. This is fortunate for those who desire to make potent commercial preparations because the α form is much more potent (some 30 times) than the β form. Most commercial products of estradiol contain only the α form, the β form having been eliminated.

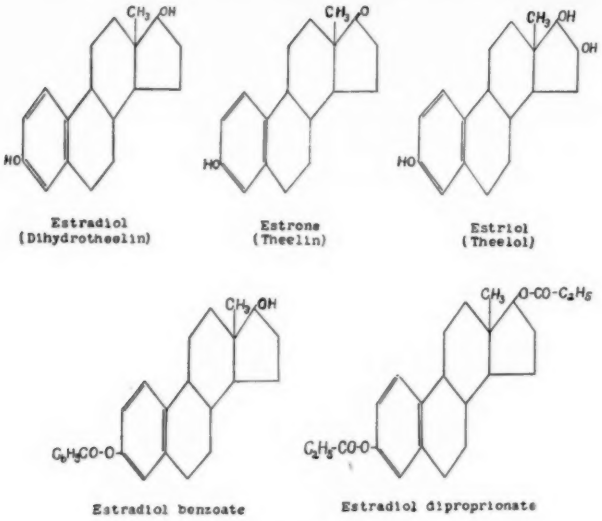
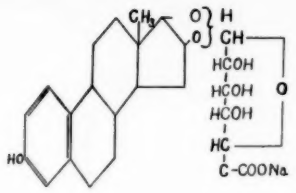


Figure 2

have been effected *in vitro* and demonstrated *in vivo*. Estradiol (dihydrotheelin) is the only one which has been actually isolated from ovarian tissue, as I stated earlier, and the activity of the follicular fluid is in a large measure accounted for by the estradiol content. The remaining fraction is ketonic in nature and is probably estrone. Estrone and estriol have been isolated from the urine of normal and pregnant women and from certain tissues of the body. These findings, in themselves, indicate that estrone and estriol may be the metabolites of estradiol. Further evidence supporting this view was furnished by Westerfeld and Doisy[19] and by Pincus and Zahl.[20] They demonstrated the *in vivo* conversion of the hormones after injections into rabbits and monkeys.

The site of conversion or metabolism of the estrogen in the body is still uncertain. Pincus and Zahl claim that the conversion of estrone to estriol in the rabbit requires a functional uterus, for they could not demonstrate this conversion in hysterectomized or long-time castrated animals. Westerfeld and Doisy,[19] however, found that the presence or absence of the uterus had no effect on the conversion of estradiol to estrone and vice versa. Whether or not these two sets of experiments deal with two different mechanisms is not yet known.



Estriol glucuronidate

Figure 3

The urinary estrogens are largely excreted in a combined form, as is indicated by the discovery that yields of estrogen from urine are markedly increased by preliminary hydrolysis. Marrian and co-workers[21] have isolated the combined estriol from human pregnancy urine and found it to consist of estriol glucuronidate, the linkage involving the terminal aldehyde group of the glucuronic acid and the hydroxyl group of carbon 16 or 17 of the estriol. The complete explanation for this conjugation is not yet known. However, it is well known that glucuronic acid is used by the organism in detoxicating many substances. Also, the conjugation with glucuronic acid increases the water solubility of estriol. A third fact, demonstrated by Marrian[22] is that the glucuronidate is relatively inactive as compared with the free estriol. Thus it may be that the conjugation is made by the organism in order to detoxicate the estriol, to increase its solubility in the urine or to inactivate the estrogenic potency or for all three reasons. That the inactivation may be of particular importance during pregnancy is suggested by Marrian's finding that shortly before the onset of labor the proportion of free estrogen rises very markedly. Marrian deduced from this that possibly the increase in free estrogen is for the purpose of sensitizing the uterus to the oxytocic principle of the pituitary and so to facilitate the onset of labor. During the first eight and one-half months of pregnancy there is no need for sensitization of the uterus to oxytocin and so the estriol is converted to the relatively inactive glucuronidate.

Estrone is also conjugated before excretion. Preliminary work by Schacter and Marrian[23] suggests that in pregnant mares' urine, estrone is present in combination with sulphuric acid. Whether or not this is also true for the estrone of human pregnancy urine is not yet known.

The nature of the estrogen conjugates of normal human urine is likewise unknown.

The site in the body where the estrogens are conjugated and so rendered inactive appears to be the liver. Zondek,[24] a number of years ago, showed that estrogen was inactivated when incubated *in vitro* with liver. Israel and his colleagues[25] found inactivation to occur in a lung-heart-liver perfusion system, but not in a lung-heart preparation. Golden and E. L. Sevringhaus[26] noted that in animals in which the ovaries were transplanted into the mesentery no evidence of heat appeared, and Biskind and Mark[27] showed that pellets of estrone implanted in the spleen when the circulation was intact gave no response, whereas if the splenic artery and vein were ligated the pellets were effective, presumably because of absorption by the non-portal part of the circulation. Although a role of the altered spleen cannot be overlooked in the inactivation of the estrone in these last experiments, nevertheless the evidence from these various experimental approaches seems definitely to show that the conjugation and consequent inactivation of the estrogens takes place in the liver. If an estrogen is always destroyed in its passage through the circulation of the liver, however, it is puzzling to understand how this substance, when given by mouth, can be effective. Estriol, it will be recalled, is quite effective by mouth in experimental animals, at least. Another puzzling finding is that of Dingemanse et al,[28] who have recently reported that considerable amounts of combined estrogen are found in ovaries. It seems quite unlikely that this could have been conjugated in the liver and then stored in the ovaries, although this is a possibility. If such is not the case the alternative remains of it having been actually secreted in the ovary in a combined inactive form.

The inactivation of estrogen by an organ such as the liver may partially explain the pronounced response from percutaneous or perepithelial administration, even though the rate of absorption is low, for in this method of administration all the estrogen would act on the underlying structures before passing into the circulation and there being inactivated in its passage through the liver circulation.

The active substance peculiar to the corpus luteum, named progesterone, was isolated in 1934, simultaneously by four different laboratories. Its structure has been determined, showing it to belong to the same family of compounds as the estrogens. It is to be noted that progesterone has quite different active groupings from the estrogens (Figure

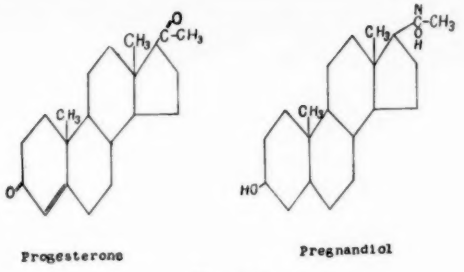


Figure 4

4). Instead of a phenolic hydroxyl on carbon 3, progesterone has a ketone group with a single unsaturated linkage in this position. On carbon 17, instead of a hydroxyl or ketone group, progesterone has an acetyl group. In addition, progesterone has the usual sterol methyl group on carbon 10. This, it will be recalled, is absent in the estrogens.

It is converted by the endometrium, according to Venning and Browne,[29] into an inactive compound, pregnandiol. These investigators report that during pregnancy and during the last half of the menstrual cycle, when luteal activity is high, pregnandiol is excreted in the urine. Similarly they find that injections of progesterone into ovariectomized women, previously sensitized by estrogen, result in pregnandiol excretion. However, no pregnandiol could be demonstrated after administration of progesterone into hysterectomized women. This led Venning and Browne[29] to postulate that the reduction to pregnandiol takes place in the endometrium. However, recent work by Buxton and Westphal[30] indicates that other mechanisms for the conversion are possible. They find that normal and pathological men, treated with progesterone, excrete almost theoretical amounts of pregnandiol. In these cases, certainly, the conversion could not take place in the uterine endometrium.

Venning and Browne have advocated the use of urinary pregnandiol determination as a measure of corpus luteum activity. That the determination has a great value is undeniable. However, it should be remembered that the pregnandiol is but the end product of the chemical reduction of progesterone. A large number of intermediate products is possible—in fact, many of them have been isolated from urine. It therefore appears that negative findings as to pregnandiol excretion need not necessarily mean lack of luteal activity. It is quite possible that pro-

gesterone may be secreted at a normal rate by the corpus luteum but that its conversion to pregnandiol is incomplete. Thus, one would find little of this compound in the urine, although a careful search might show the presence of other degradation products of progesterone. It is evident that in order to determine the presence or absence of active corpora lutea, pregnandiol determinations should be supplemented by uterine biopsies.

The Action of Estrogen

The estrogens are powerful pharmacological agents. A response of the female accessory reproductive organs was the first one noted and is the most prominent effect and so it is quite logical that it has been the one on which attention has been primarily focused, and effects on other structures neglected. The few studies which have been made on other responses make it unsafe to assume, however, that only the reproductive system, including the mammary gland, is affected by these hormones. These remarks could be justified even though no relationship to the genesis of cancer had been established. The reaction induced by the various estrogens is similar, differing only in intensity and duration, and so can here be treated as due to a single substance.

The action of estrogen on the uterus is well known and need be only briefly discussed. The uterus after ovariectomy undergoes profound involution; the glands become short and few in number and the epithelium of a low non-secretory type. If estrogen is administered all parts of the organ are stimulated. The myometrium becomes thicker and more vascular. The endometrium particularly responds, becoming thickened and showing a growth in the number and length of its glands, and some glycogen deposition takes place in the basal zone of the gland cells. The epithelium is increased in height. The vascular response is immediate, pronounced and striking, as shown by direct observation of the uterus in rabbits through an abdominal window and of endometrial transplants in the anterior chamber of the eye (Markee,[31] Pompen[32]). There is an early increase in the acetylcholine content, as shown by the fact that atropine will inhibit the dilatation of the vascular bed and by tests on eserinized frog muscle (Pompen,[32] Reynolds[33]). The endometrium imbibes fluid from the dilated and slowed circulation. These vascular changes precede the later enhanced growth and metabolic changes. The resultant effect is then a proliferation of epithelial cells and a thick-

ened and a highly vascular and edematous endometrium. For the development of the type of endometrium in which implantation of the fertilized egg can take place, the second female sex hormone, progesterone, is necessary. Although its first action, as shown by experimental work, is to increase mitoses in the glands and epithelial lining of the uterus (Lloyd[34]), its later and specific effect is to bring about a secretory condition in this epithelium. Glycogen, which lies in the basal portion of the gland cells in the estrogen phase, is transferred to the apical zone and together with mucus is secreted into the lumina of the glands. The lumina become not only filled with this secretion but become enlarged and irregular in shape.

Although the induction of this secretory or progravid state of the endometrium is a specific action of the corpus luteum hormone, its action is enhanced by estrogen, that is, the amount of progesterone necessary to produce a progravid endometrium is decreased by injecting estrogen (Hisaw et al,[35]). Engle and Smith[36]).* Not only is the progesterone response enhanced by estrogen, but, as has been experimentally shown, the progravid condition can be maintained for a longer period if estrogen is concurrently administered. This work, which has shown that the progesterone response is enhanced by estrogen, thus correlates with what I have described earlier, namely, that the corpus luteum normally secretes both progesterone and estrogen.

* In earlier work (Hisaw and Leonard,[37] Clauberg,[38] Robson[39]) on the combined action of estrogen and progesterone on the endometrium of rabbits, it was found that the progestational effect of progesterone was nullified by the concurrent injection of relatively small amounts of estrogen. Estrogen is pronouncedly antagonistic to progesterone in rabbits and the progestational action of the latter is nullified if about 1/75 to 1/100 of its weight of estrone is administered, or in international units, a ratio of about 1 to 100. However, even in rabbits the addition of some estrone to the progesterone after a progravid condition had been developed by progesterone alone, prolongs the progravid condition (W. M. Allen[40]). Normally the progravid condition induced by progesterone administration in spayed rabbits disappears by the tenth day. In order to secure a progesterone response it is necessary, of course, to sensitize the uterus by a preceding series of estrogen injections.

In monkeys the estrogen-progesterone ratio which will prevent the progestational reaction is much higher than in rabbits. A daily dosage of estrogen as high as 500 R.U. or approximately 2500 to 5000 I.U. does not inhibit the action of .5 I.U. of progesterone, a ratio in I.U. of 5000-10,000 to 1 (Allen, Hisaw and Gardner[41]). These findings in monkeys appear to correlate much better with those in the human than does the work on rabbits, although no precise experiments have been reported in the human. Not only is it well known that corpora lutea in the human contain much estrogen, but in addition assays of blood and urine show a much larger amount of estrogen than can be found in lower forms, including monkeys. It seems safe to assume that the action of the progesterone from the human ovary is increased and not inhibited in the human by the circulating estrogen.

I would like to point out, however, that the response of the endometrium does not depend solely on the action of estrogen and progesterone, but is influenced by altered body states. In hypophysectomized monkeys we have found that the response to these hormones is less than in spayed animals and that the latent period between withdrawal of both estrogen and of progesterone and menstruation is very greatly increased. The cachexia and other disturbances induced by hypophysectomy evidently reduce the quantitative responses to these hormones, although qualitatively their actions are not changed.

Curious as it may seem, work has established that estrogen assists in the structural and presumably the functional maintenance of the corpus luteum itself. I refer to the work of Westman[42] and of Robson[43] in rabbits in which it was shown that the rapid regression of the corpora which characteristically occurs after hypophysectomy can be prevented by the administration of estrogen. In such an experiment it is obvious that this maintenance cannot be indirectly due to a stimulation of the hypophysis. Although an indirect effect through other endocrine glands has not been disproven, nevertheless this work indicates that estrogen, an ovarian secretion, assists in the maintenance of an ovarian structure, the corpus luteum. This could justifiably be characterized as a gonadotropic effect of estrogen. This corpus luteum maintenance effect of estrogen, however, is not permanent and regression of the corpora ultimately occurs, even though the injection of estrogen is continued. Regression which would normally occur within five days is postponed only until the thirteenth to the sixteenth day, which is the period that corpora lutea remain functional in pseudopregnancy in rabbits. We have to admit that the reactions of the reproductive organs of rabbits are peculiar in some respects and that this gonadotropic effect of estrogen may not hold in monkeys and the human. The presumption is, however, that the same response will be found in these higher forms.

It seems desirable to mention some of the hormonal conditions obtaining in pregnancy, for not only is the maintenance of the state of pregnancy dependent on the continued secretion of the ovaries in most forms, but the newly formed organ, the placenta, is a secretory organ and supplies hormones normally secreted primarily by the ovary. In most laboratory animals abortion soon takes place, when the ovaries are removed. Such is not the case in the human, in monkeys, in guinea pigs and in mares. Except in monkeys, the literature is sufficiently extensive

and definite to establish that the ovaries are not essential for the continuation of pregnancy in these species.

A number of years ago Robert Frank pointed out that the placenta probably had a secretory function, and this has been found to be the case. It appears to be highly probable, and indeed established, that the gonadotropic hormone which appears in the urine in large amounts early in pregnancy in women is formed by the placenta. The designation of this hormone as chorionic gonadotropin is being widely adopted and this name was accepted at the Third International Conference on the Standardization of Hormones held in Geneva last year. Estrogen continues to be present after the removal of the ovaries during pregnancy, although further studies are desirable to determine if it is in normal amounts and how great a reduction invariably occurs after ovariectomy. The placenta contains a higher concentration of estrogen, mostly as estriol, than any other organ of the body.

Less work has been done on the placental source of progesterone than is the case with the estrogens. A method for the assay on the excreted degradation products of progesterone has only recently been devised, and though it has already proven of great value in supplying additional evidence on the period when progesterone is secreted in the normal cycle and during pregnancy, nevertheless but little work on the effect of ovariectomy on the secretion of progesterone in pregnant women has been done. Browne et al[44] and Jones and Weil[45] have reported pregnandiol excretion after excision of the corpus luteum during pregnancy. In the case of Jones and Weil there was a temporary drop followed by a return to nearly normal levels. Even though data do not conclusively show that the placenta secretes estrogen and progesterone, it appears highly probable that such is the case and that in the special condition of pregnancy the placenta supplements and indeed can take over in the human the secretion of the female sex hormones in the absence of the ovaries.

I shall not include a discussion of the effects of estrogen and progesterone on the two other main structures of the reproductive system, the vagina and mammary glands. Proliferation of the epithelial elements of both organs is caused by estrogen. In the vagina there is an increased deposition of glycogen and a consequent lowering of the pH. This increased acidity and accompanying epithelial proliferation and cornification is probably responsible for the benefit in gonorrheal vaginitis.

As stated earlier in the paper, the extra-reproductive effects of estrogen have not been investigated nearly as extensively as have the responses of the reproductive organs. Studies, it is true, have been made on such subjects as the influence of estrogen on ketosis, carbohydrate, fat and basal metabolism, serum calcium, and blood formation, but not only is more work required before significant or at least definite conclusions, in most cases, can be drawn, but also any indirect effect through the pituitary gland must be further tested, so that it would be premature to attempt to draw conclusions at this time.

Although blood pressure determinations after estrogen injections are negative and consequently an effect on the larger blood vessels and the heart is improbable, nevertheless significant and definite changes have been reported in the peripheral circulation. Work on the peripheral vessels was stimulated from two findings. First, injection of estrogen causes within an hour in rabbits a pronounced hyperemia. This is not due to a heightened metabolism of the tissue, for the hyperemia precedes an increase in oxygen consumption. Secondly, the hyperemia from estrogen injection is not limited to the uterus but is participated in by the sexual skin in monkeys and by the nasal mucosa. The hyperemia persists throughout the estrogen injection and leads at least in the uterus and the sexual skin of monkeys to water retention and a high osmotic pressure of the interstitial fluid (Aykroyd and Zuckerman[46]) and sodium retention (Thorn and Harrop[47]). In a form in which the sexual skin changes are pronounced, the water retention is sufficient to produce a significant increase in body weight (Krohn and Zuckerman[48]). The weight increase is accompanied by a decrease in urine output. Although a change in the water content of the skin is more pronounced in forms having a distinct swelling of the sexual skin, nevertheless an effect on water retention from estrogen injections has also been shown to occur in rats, a species in which there is no external manifestation of changes in the skin (Zuckerman et al[49]). This shift in water to the skin in rats amounts to almost 1 per cent of the body weight and is accompanied by slight decrease in the water content of muscle and certain of the organs. This water retention effect of estrogen is also induced by other sterol hormones, although certain differences, as in nitrogen retention, occur and Zuckerman[50] states that the excretion of water which has accumulated in the sex skin of monkeys from estrogen administration is not prevented by progesterone, testosterone or cortin. The effect of estrogen

on the peripheral circulation has been further explored by Reynolds and his co-workers in rabbits and the human. By direct observation on the transilluminated ear of rabbits Reynolds and Foster[51, 52] observed that injection of estrogen caused a dilatation of the capillaries and small venules. The ear became pink. That there was usually not an increased blood flow was evidenced by a fall rather than a rise in the temperature of the ear. The effect was limited solely to the small peripheral vessels as there was no constant change in blood pressure. In measurement of the volume of the finger in men they (Reynolds and Foster[53]) found an increase which averaged 4.6 per cent in twelve subjects, whereas in six there was no response. Although the increase was small, attention was called to the fact that the fleshy part of a finger comprises only about one-half the total volume of this digit, and an increase in size would be limited to the soft part and not to the hard part—the nail and bone. Although work on the peripheral vascular responses has not been extensive, yet sufficient work appears to have been done to establish that vascular effects are not limited to the sexual organs of the female.

The mechanism by which estrogen alters the peripheral vascular bed, so far as it has been elucidated, seems to be an interesting one. Pompen[32] found that the heightened vascularity of the uterus induced by estrogen injections would be inhibited by atropine sulphate. This indicated that the response might be due to an increase in acetylcholine. Reynolds[51] has carried out a series of assays of the acetylcholine content of the uterus. He finds that in ovariectomized rabbits no trace of this substance could be found in most cases, whereas within an hour after an injection of an estrogen (Amniotin, Squibb) a very appreciable amount was present. Whether, in the general peripheral vascular bed, acetylcholine is the agent responsible for the dilatation of the small vessels remains yet to be established. That such may be the case is indicated by the interesting findings reported by Soskin and Bernheimer[54] in atrophic rhinitis. These investigators postulated that the beneficial effects in atrophic rhinitis reported by Mortimer et al[55] and by Blaisdell,[56] from injections of estrogen, were due to hyperemia. They tested this hypothesis not by local application of acetylcholine, which might be dangerous, but by potentiating that which was normally present but which is inactivated by choline esterase. They inhibited the action of the latter with local applications of a physostigmin-like substance. In a moderate-sized series, fourteen patients, this treatment, they report, gave better

results than were secured with local applications of estrogen. If further work confirms this report, it will be an excellent example of an immediate practical application of findings which appeared to be of theoretical physiological interest only.

Among the extragonadal effects of estrogen the action on the anterior pituitary gland is outstanding. This action is so well known, however, that a brief discussion will suffice.

It was early noted that the daily subcutaneous injection of estrogenic preparations damaged the reproductive apparatus of male animals. At first this was attributed to a hormone antagonism but when it was later found that ovarian atrophy also resulted, it seemed likely that the injury was due to decreased hypophyseal activity. This view was strengthened by the discovery that the injection of gonadotropic hormone concurrently with estrogen prevented the ovarian atrophy in normal animals and that in hypophysectomized rats estrogen administration did not decrease the effect of gonadotropic hormones. The physiological evidence seemed to show clearly a depressant effect on the hypophysis. Evidence soon appeared, however, showing in certain but not in all forms that estrogen caused a formation of corpora lutea, a finding which would indicate an enhanced output of luteinizing hormone, according to the ideas current about the two hypophyseal gonadotropic hormones. There was thus suggested a reciprocal relationship between the ovaries and the hypophysis, which would very nicely explain the rhythmicality of the female sex cycle. With the growth of the follicles in the first half of the cycle there would be increasing secretion of estrogen which in turn would depress the output of follicle-stimulating hormone and increase the secretion of luteinizing hormone. As the correct balance between these two gonadotropic hormones was reached, rupture of the follicles would result and corpora lutea would form and be maintained by the luteinizing hormone. Such a concept, as shown by subsequent work, is far too simple. Luteinizing A.P. hormone, as prepared by present methods, does not maintain the corpora lutea, but rather in some forms, at least, it causes their disappearance. The lactogenic hormone, on the other hand, induces the functional and structural persistence of the corpora lutea. The ovarian-hypophyseal interrelationship in the cycle is apparently not as simple as was outlined. The marshalling of additional data frequently spoils these simple *schemata*.

However, both experimental and clinical work show that estrogen,

when given in considerable amounts, represses the liberation of at least one of the two or more gonadotropic hormones supposed to be secreted by the hypophysis. Even this finding, which seems clear from all physiological evidence, is difficult, however, to harmonize with the structural changes which occur in the hypophysis, for these changes definitely indicate an increased activity of the basophiles. However, it is certain that the administration of estrogen decreases the urinary output of pituitary gonadotropic hormone which normally rises at the menopause, a rise which has been given as the cause of the menopausal disturbances. This may well be too simple an explanation, for the influence of estrogen is not limited to the hypophysis and the reproductive system as pointed out earlier in this paper.

The repressing effect of estrogen on the hypophysis is not limited to the gonadotropic hormone or hormones. A number of experimental studies have shown a slowing or indeed a stoppage of the growth in young animals. Although there are differences of interpretation in regard to the changes in the epiphyseal cartilages from estrogen administration (Tausk and de Fremery,[57] Zondek,[58] Pfeiffer and Gardner,[59] Silberberg and Silberberg[60]), it can be stated that, whatever may be the nature of these changes, it is certain an abrupt stoppage of growth can be induced in experimental animals (Deanesly[61] and others). The injection of estrogen, however, could hardly be recommended as a method for limiting growth in the human, because the effects of this hormone are general, as I have emphasized earlier in this paper. In addition to the depressant effects on growth, we can add the more questionable one of depression of the thyroid and the induction of an adrenal hypertrophy.

In this paper I have limited myself to a discussion of the action of the ovarian hormones, for this subject is sufficiently complex to make me well-satisfied to have had the pituitary gonadotropic hormones and their action on the ovaries discussed earlier in the Fortnight series by J. B. Collip. I may, perhaps, have appeared to overemphasize the complexity of the action of estrogenic hormones, although I believe such is not the case. I have tried to point out that the estrogens—certainly much more so than progesterone—are powerful pharmacological agents. Even though we overlook the possibility of carcinogenic effects, they cannot be given with assurance that their action will be circumscribed or limited. The advance in our knowledge about the action of the female sex hormone has been rapid, for it is only in the last few years that these substances

have been made available in quantities sufficient to carry on adequately experimental and clinical investigation with them. Since these hormones are powerful pharmacological agents and have a widespread action on the body, it is important to keep in mind that they should not be promiscuously given and that it is important to know when they should not be used as well as to know when they may be of value.

REFERENCES

1. Schroeder, R. Die weiblichen Genitalorgane, in *Handbuch der mikroskopischen Anatomie des Menschen* (von Möllendorff). Berlin, Springer, 1930, v. *7*, pt. 1, p. 329.
2. Allen, E. Ovogenesis during sexual maturity, *Am. J. Anat.*, 1922-23, *31*, 439.
3. Evans, H. M. and Swezy, O. Ovogenesis and the normal follicular cycle in adult mammalia, *Memoirs Univ. California*, 1931, *9:* no. 3.
4. Kingsbury, B. F. Postpartum formation of egg cells in the cat, *J. Morphol.*, 1938, *63:* 397.
5. Allen, E., Doisy, E. A. *et al.* The hormone of the ovarian follicle, *Am. J. Anat.*, 1924-25, *34:* 133.
6. Corner, G. W. Sites of formation of estrogenic substances in the animal body, *Physiol. Rev.*, 1938, *18:* 154.
7. Gallagher, T. F., Peterson, D. H., Dorfman, R. I., Kenyon, A. T. and Koch, F. C. The daily urinary excretion of estrogenic and androgenic substances by normal men and women, *J. Clin. Investigation*, 1937, *16:* 695.
8. Dingemanse, E., Borchardt, H. and Laqueur, E. Capon comb growth-promoting substances ("male hormones") in human urine of males and females of varying ages, *Biochem. J.*, 1937, *31:* 500.
9. Callow, R. K. Significance of the excretion of sex hormones in the urine, *Proc. Roy. Soc. Med.*, 1938, *31:* 841.
10. Hill, R. T. Ovaries secrete male hormone; restoration of castrate type of seminal vesicle and prostate glands to normal by grafts of ovaries in mice, *Endocrinology*, 1937, *21:* 495.
11. Deanesly, R. Androgenic activity of ovarian grafts, *J. Physiol.*, 1938, *92:* 34*P*.
12. Hamblen, E. C. *et al.* Studies of the metabolism of androgens in women, *Endocrinology*, 1939, *25:* 491.
13. Hirschmann, H. Androgens from the urine of ovariectomized women, *J. Biol. Chem.*, 1939, *130:* 421.
14. Callow, N. H. and Callow, R. K. Isolation of 17-ketosteroids from the urine of normal women, *Biochem. J.*, 1939, *35:* 931.
15. Frank, R. T., Goldberger, M. A. and Spielman, F. Present endocrine diagnosis and therapy; critical analysis based on hormone studies in females, *J.A.M.A.*, 1934, *103:* 393.
16. Fluhmann, C. F. Estrogenic substance in the blood of women, *Am. J. Obst. & Gynec.*, 1936, *32:* 612.
17. MacCorquodale, D. W., Thayer, S. A. and Doisy, E. A. The isolation of the principal estrogenic substance of liquor folliculi, *J. Biol. Chem.*, 1935, *115:* 435.
18. Bachmann, H. E., Cole, W. and Wilds, A. L. The total synthesis of the sex hormone equilenin, *J. Am. Chem. Soc.*, 1939, *61:* 974.
19. Westerfeld, W. W. and Doisy, E. A. Ketonic and non-ketonic estrogens, *Ann. Int. Med.*, 1937, *11:* 267.
20. Pincus, G. and Zahl, P. A. Biogenesis of primary sex hormones; fate of estrine injected into the rabbit, *J. Gen. Physiol.*, 1937, *20:* 879.
21. Cohan, S. L., Marrian, G. F. and Odell, A. D. Oestriolglucuronide, *Biochem. J.*, 1936, *30:* 2250.
22. Marrian, G. F. The conjugated estrogens, *Cold Spring Harbor Symposia on Quantitative Biology*, 1937, *5:* 16.
23. Schacter, B. and Marrian, G. F. Observations on conjugated oestrogens in the urine of pregnant mares, *Proc. Soc.*

Exper. Biol. & Med., 1936-37, *35:* 222.

24. Zondek, B. Über das Schicksal des Follikelhormons (Follikulin) im Organismus, *Skandinav. Arch. f. Physiol.*, 1934, *70:* 133.
25. Israel, S. L., Meranze, D. R. and Johnston, C. G. Inactivation of estrogen by liver, *Am. J. M. Sc.*, 1937, *194:* 835.
26. Golden, J. B. and Sevringhaus, E. L. Inactivation of estrogenic hormone of the ovary by the liver, *Proc. Soc. Exper. Biol. & Med.*, 1938, *39:* 361.
27. Biskind, G. R. and Mark, J. Inactivation of testosterone propionate and estrone in rats, *Bull. Johns Hopkins Hosp.*, 1939, *65:* 212.
28. Dingemanse, E. T. and Mühlbock, O. Über die freien und gebundenen oestrogenen Hormone im Ovarium des Pferdes, der Kuh und des Schweines, *Acta brev. Neerland.*, 1939, *9:* 95.
29. Venning, E. H. and Browne, J. S. L. Studies on corpus luteum function; urinary excretion of sodium pregnandiol glucuronidate in the human menstrual cycle, *Endocrinology*, 1937, *21:* 711.
30. Buxton, C. L. and Westphal, U. Recovery of pregnandiol in urine of men treated with progesterone, *Proc. Soc. Exper. Biol. & Med.*, 1939, *41:* 284.
31. Markee, J. E. Rythmic vascular uterine changes, *Am. J. Physiol.*, 1932, *100:* 32.
32. Pompen, A. W. M. *The influence of menformon on the uterus.* Amsterdam Thesis, 1933.
33. Reynolds, S. R. M. Acetylcholine content of uteri before and after administration of oestrin to ovariectomized rabbits, *J. Physiol.*, 1939, *95:* 258.
34. Lloyd, C. W. Effect of progesterone on cell-division in uterine epithelium, *Proc. Soc. Exper. Biol. & Med.*, 1937, *36:* 190.
35. Hisaw, F. L., Greep, R. O. and Fevold, H. L. Effects of estrin-progestin combinations on endometrium, vagina and sexual skin of monkeys, *Am. J. Anat.*, 1937, *61:* 483.
36. Engle, E. T. and Smith, P. E. Endometrium of the monkey and estrone-progesterone balance, *Am. J. Anat.*, 1938, *63:* 349.
37. Hisaw, F. L. and Leonard, S. L. Relation of follicular and corpus luteum hormones in the production of progestational proliferation of rabbit's uterus, *Am. J. Physiol.*, 1930, *92:* 574.
38. Clauberg, C. Das Hormon des Corpus luteum, *Zentralbl. f. Gynäk.*, 1930, *54:* 7.
39. Robson, J. M. Action of progesterone on the uterus of the rabbit and its antagonism by oestrone, *J. Physiol.*, 1936, *88:* 100.
40. Allen, W. M. and Heckel, G. P. The effect of continued injections of progestin and combinations of oestrin and progestin on the endometrium of the castrated rabbit, *Anat. Rec.*, 1935-36, *64*, suppl. 3: 2.
41. Allen, E., Hisaw, F. L. and Gardner, W. U. The endocrine functions of the ovaries, in *Sex and internal secretions.* Baltimore, Williams and Wilkins, 2. ed., 1939, chapt. 8, p. 554.
42. Westman, A. and Jacobsohn, D. Über Oestrinwirkungen auf die Corpus luteum-Funktion, *Acta obst. et gynec. Scandinav.*, 1937, *17:* 1; 13.
43. Robson, J. M. Rôle of gonadotropic hormone in the maintenance of luteal function, *Quart. J. Exper. Physiol.*, 1938, *28:* 49.
44. Browne, J. S. L., Henry, J. S. and Venning, E. M. The corpus luteum hormone in pregnancy, *J. Clin. Investigation*, 1937, *16:* 678.
45. Jones, H. W. and Weil, P. G. Corpus luteum hormone in early pregnancy; report of case in which there was early removal of the corpus luteum, *J.A.M.A.*, 1938, *111:* 519.
46. Aykroyd, O. E. and Zuckerman, S. Factors in sexual-skin oedema, *J. Physiol.*, 1938, *94:* 13.
47. Thorn, G. W. and Harrop, G. A. "Sodium retaining effect" of sex hormones, *Science*, 1937, *86:* 40.
48. Krohn, P. L. and Zuckerman, S. Water metabolism in relation to the menstrual cycle, *J. Physiol.*, 1937, *88:* 369.
49. Zuckerman, S., Palmer, A. and Bourne, G. Changes in water-content of organs and tissues as a result of stimulation by œstradiol, *Nature*, 1939, *143:* 521.
50. Zuckerman, S. Water-retention in the

reproductive organs of female monkeys, *J. Physiol.*, 1938, *94:* 3P.

51. Reynolds, S. R. M. and Foster, F. I. Peripheral vascular action of oestrin in the rabbit and the human, *Am. J. Physiol.*, 1939, *126:* P606.
52. Reynolds, S. R. M. and Foster, F. I. Peripheral vascular effects of estrogen observed in the ear of the rabbit, *J. Pharm. & Exper. Therap.*, 1940, *68:* 173.
53. Reynolds, S. R. M. and Foster, F. I. Peripheral vascular action of estrogen in the human male, *J. Clin. Investigation*, 1939, *18:* 649.
54. Soskin, S. and Bernheimer, L. B. Mechanism of estrogen effect on nasal mucosa in atrophic rhinitis; successful treatment with prostigmin, *Proc. Soc. Exper. Biol. & Med.*, 1939, *42:* 223.
55. Mortimer, H., Wright, R. P. and Collip, J. B. Atrophic rhinitis; constitutional factor; and treatment with oestrogenic hormones, *Canadian M. A. J.*, 1937, *37:* 445.
56. Blaisdell, I H. Use of estrogenic substances in atrophic rhinitis, *Laryngoscope*, 1938, *48:* 699.
57. Tausk, M. and de Fremery, P. Über den Einfluss des Follikelhormons (Menformon) auf die Ossifikation bei kastrierten Hunden, *Acta brev. Neerland.*, 1935, *5:* 19.
58. Zondek, B. Impairment of anterior pituitary functions by follicular hormone, *Lancet*, 1936, *2:* 842.
59. Pfeiffer, C. A. and Gardner, W. U. Skeletal changes and blood serum calcium level in pigeons receiving estrogens, *Endocrinology*, 1938, *23:* 485.
60. Silberberg, M. and Silberberg, R. Action of estrogen on skeletal tissues of immature guinea pigs, *Arch. Path.*, 1939, *28:* 340.
61. Deanesly, R. Depression of hypophyseal activity by implantation of tablets of oestrone and oestradiol, *J. Endocrinol.*, 1939, *1:* 36.

THE BIOLOGICAL SIGNIFICANCE OF NICOTINIC ACID

Harvey Lecture, November 16, 1939

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IF we review briefly the developments in the field of biochemistry, we find that chemical elements and chemical compounds have become biologically significant by diverse pathways. The various methods by which the five recognized members of the B complex have come to be known as specific chemical compounds makes an interesting story in itself. In the case of riboflavin the new compound was isolated and later associated with fundamental processes in the living cell. In the other cases the necessity of compounds of specific character was recognized first and finally the actual compounds were isolated and identified. Thiamin, vitamin B_6, and pantothenic acid turned out to be compounds of rather unique structure, quite different from those generally found in living matter. On the other hand, the antipellagra factor was found to be a compound which had been known for many years.

It is quite obvious that in all cases our knowledge of the biological significance of these factors has increased more rapidly after the compounds became available in pure form. Thus we are not so interested in the manner in which the significance was recognized as we are in the entire and complete picture. It is my purpose this evening to give you as complete a picture as I can of the facts concerning the third member of the vitamin B complex—nicotinic acid.

Huber[1] first prepared nicotinic acid in 1867 by the oxidation of nicotine. Its isolation from biological material was not achieved until 45 years later when Funk[2] isolated nicotinic acid in crystalline form from yeast concentrates which possessed antineuritic activity. The acid itself, however, displayed no activity in curing pigeon beriberi. At about the same time Suzuki, Shimamura, and Odake[3] isolated nicotinic acid from rice polishings. In 1916 Williams,[4] impressed by the common occurrence

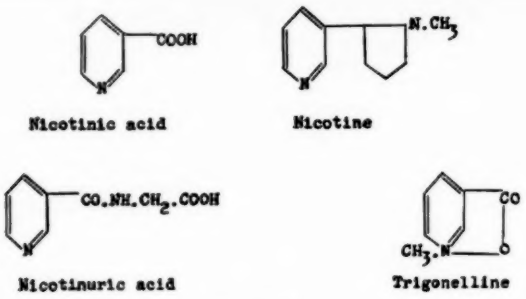

Figure 1

of nicotinic acid with the antineuritic vitamin in several natural substances, again tried nicotinic acid, trigonelline, as well as other pyridine derivatives for antineuritic potency, but none of them caused any permanent improvement in polyneuritic fowls. Trigonelline, the methyl betaine of nicotinic acid, was isolated from plant material by Jahns[5] in 1885. In 1912 Ackermann[6] found that dogs given fairly large amounts of nicotinic acid excreted in the urine about equal amounts of trigonelline and nicotinuric acid (the dipeptide of nicotinic acid and glycine). The formulæ for these compounds are given in Fig. 1.

Except for the work of Szymanska and Funk[7] who attributed an appetite-stimulating and weight-preserving action to nicotinic acid and the amide, very little interest was shown in the possible role of pyridine derivatives in living systems until the work of Warburg and Euler in 1935. Warburg and Christian[8] characterized nicotinic acid amide as one of the hydrolysis products from the coenzyme which they had isolated from blood and which is now known as coenzyme II. Kuhn and Vetter[9] isolated nicotinic acid amide from heart muscle and Euler, Albers, and Schlenk[10] from cozymase.

This work gave new impetus to the application of these compounds in the field of nutrition. Euler and Malmberg,[11] using a diet similar to the Sherman-Bourquin diet supplemented with vitamin B_1 and riboflavin, found no growth response with the acid or amide although the rats receiving the acid lived longer. Funk and Funk[12] found larger food intake and better growth in rats and pigeons on certain rations when given the acid and especially the amide. In our own laboratory[13] we observed a growth stimulus from nicotinic acid when fed with adenylic

acid to rats on a factor W deficient diet. However, none of these responses was of sufficient magnitude to attribute to nicotinic acid real vitamin-like properties.

Let us now turn our attention to the human disease, pellagra. Almost twenty years ago Dr. Voegtlin presented before this Society the studies on pellagra available at that time and concluded that the disease could be prevented by appropriate diet, but the nature of the dietary factor had not been discovered. By 1930, due to the excellent work of Goldberger and co-workers, pellagra had been definitely established as a deficiency disease and the protective factor was associated with the more heat stable fraction of the B complex which had been distinguished from vitamin B_1 by animal experimentation. A pellagra-like condition had been produced in rats on diets low in the B complex supplemented with an alcoholic extract of corn. I want to emphasize at this time that Goldberger never referred to this syndrome as anything except a pellagra-like condition. The symptoms observed in rats by Goldberger were undoubtedly those which we associate today with vitamin B_6 and riboflavin deficiency. Considerable difficulty was encountered in producing this condition consistently but the growth-stimulating effect of various foods when added to this diet continued to be used as a measure of antipellagra activity. All the values in the literature were based on these assays except the limited figures reported by Sebrell[14] from studies with dogs.

In 1930 the value of liver extract in the treatment of pellagra in humans as well as its activity in the prevention of vitamin B_2 deficiency in rats was recognized. Goldberger and Sebrell[15] found liver extract 343 to be a good source of the factor necessary for the prevention of black tongue in dogs. Spies[16] and Smith and Ruffin[17] found that fairly large amounts of liver extract by mouth were efficacious in treating pellagra. At about the same time I was working in the Biochemical Laboratory, Cambridge, England, and Dr. Guha, who was also working there, found the liver extract, which I had brought along for another purpose, produced excellent growth in rats on a vitamin B_2 deficient diet. The following year Salmon and Guerrant[18] found liver extract to be four times as rich in vitamin B (B_2) as a sample of brewers' yeast. We now know that the growth obtained in both cases must have been due mainly to the riboflavin present in the liver extract. However, the above results were sufficient to convince me that liver extract might be an excellent material for the isolation of the antipellagra factor.

Attempts in our laboratory to produce pellagra-like lesions in rats failed completely and we turned our attention to chicks. A pellagra-like condition was produced in the chick by feeding a heated natural grain ration, and the assays of our fractions from liver extract depended upon the prevention of these lesions. We[19] soon had the first indication that the factor active in this syndrome was separate from riboflavin which Kuhn, György, and Wagner-Jauregg[20] had just isolated and shown to have growth-promoting properties in rats. Concentrates of riboflavin were completely inactive for the chick while purified fractions from liver retained their potency after removal of riboflavin. Lepkovsky and Jukes[21] soon confirmed our experimental results and introduced the name "filtrate factor" for the active substance in the filtrate after removal of the riboflavin with fuller's earth. This designation did not exactly please us since naturally we felt that we were dealing with the true antipellagra factor, but both Dr. Lepkovsky and I recognized that there was no evidence available to show that the pellagra-like condition in chicks was identical with human pellagra. It was therefore necessary for us to repeat our work using dogs, since most authorities agreed that black tongue in dogs was identical with human pellagra. Again[22] riboflavin had no effect on black tongue while the purified concentrates free of riboflavin were highly active in curing this syndrome. Birch, György and Harris[23] confirmed the fact that riboflavin had no curative action in dogs and Dann,[24] Spies,[25] and Fouts, Lepkovsky, Helmer, and Jukes[26] reported complete inactivity of riboflavin in the treatment of human pellagra.

Further purification of the liver fractions gave concentrates which contained very small amounts of solid material and which were highly active in both chicks and dogs. It was not surprising that we believed that the chick and dog required the same factor. Within a very short period we[27] demonstrated the activity of nicotinic acid in the cure of black tongue, and isolated nicotinic acid amide from liver extract concentrates. The activity of nicotinic acid in the treatment of black tongue was soon verified by Street and Cowgill,[28] Dann,[29] and Sebrell and coworkers.[30] However, when we[31] tried nicotinic acid or the amide on chicks we found these compounds completely inactive in the prevention of the pellagra-like lesions in this species. The chicks had provided a means of assay not because we were actually testing for nicotinic acid but because we were testing for the chick antidermatitis factor which followed the nicotinic acid amide very closely. Recent work in our

laboratory conducted by Dr. Woolley has identified the chick antidermatitis factor as pantothenic acid.

The activity of nicotinic acid in the treatment of black tongue suggested its therapeutic use in human pellagra and its successful use has been reported by a number of workers in the field. The value of nicotinic acid has recently been reviewed by Spies, Bean, and Ashe[32]. They make the following summary concerning its use. "In cases of acute or chronic pellagra in relapse it will: (a) cause fading of the fiery red lesions of the mucous membranes and diminish the Vincent's infection associated with it, (b) in most cases, restore to normal disturbed gastrointestinal function, (c) restore to normal the mental function deranged moderately or severely in acute pellagra, (d) cause fading of the dermal erythema but not cure chronic changes of the skin. In cases of subclinical pellagra, the vague ill-defined symptoms disappear and in persons subject to recurrences of the disease the development of clinical pellagra is prevented. In both clinical and subclinical pellagra, the sense of well-being, one of the attributes of health, is restored."

Very recently Cleckley, Sydenstricker, and Geeslin[33] have reported the beneficial effect of nicotinic acid in the treatment of atypical psychotic states.

Katzenellenbogen[34] working in Palestine brought about considerable improvement in twenty-one out of twenty-four cases of stomatoglossitis, characterized by soreness of the tongue and angles of the mouth and sore throat. Landor,[35] however, could not cure stomatitis with nicotinic acid but did find yeast to be active. It is quite possible that these conditions may be related to the cheilosis which Sebrell[36] has shown to be due to a riboflavin deficiency.

Nicotinic acid has also been used in the treatment of disorders related to black tongue in dogs and certain intestinal disturbances in pigs. It is safe therefore to conclude that diets low in nicotinic acid or compounds which yield nicotinic acid on ingestion allow the development of pellagra and related disturbances in humans and similar conditions in dogs, pigs and monkeys.

The above results raise certain pertinent questions: (a) What is the distribution of nicotinic acid or nicotinic acid precursors in natural foods? (b) Are other compounds equally as effective as nicotinic acid, and (c) How does nicotinic acid function in the animal body?

The successful use of natural foods as a source of this vitamin de-

pends upon the availability of complete figures for its distribution in a large variety of foods. Possible methods for determining the distribution of nicotinic acid include chemical procedures, bacterial growth methods, and animal assays. The method of Karrer and Keller[37] based on the color produced with 2,4 dinitrochlorbenzene is definitely not reliable since the value given by Karrer for liver is one-tenth of the amount of nicotinic acid amide which we actually isolated from liver and we calculated that we recovered only one-twentieth of that present. The method which has been most satisfactory depends upon the breakdown of the pyridine nucleus by cyanogen bromide and aniline to give a yellow colored compound which can be measured colorimetrically. Swaminathan[38] has used this method on foods and Shaw and MacDonald[39] on commercial liver extracts. Bandier and Hald[40] have used p-methylaminophenol in place of aniline, and Bandier[41] has recently applied this method to biological material with satisfactory results.

Nicotinic acid was shown to be an important growth factor for Staphylococcus aureus by Knight,[42] for diphtheria bacillus by Mueller,[43] and for dysentery bacillus by Koser, Dorfman, and Saunders.[44] Although some of these methods have been used for the quantitative estimation of nicotinic acid in body fluids, no extensive studies have been made on foods. Similarly cozymase has been determined through the use of the bacilli of the influenzæ group as originally demonstrated by Lwoff and Lwoff but as yet this method has been applied mainly to blood by Vilter, Vilter, and Spies[45] and by Kohn[46].

As far as animal assays are concerned, both the rat and the chick are eliminated at least for the time being. We have, therefore, continued to rely upon the dog for our assays. The curative method has been used almost exclusively. Black tongue was produced on our regular basal ration containing:

Yellow corn	72
Purified casein	18
Cottonseed oil	5
Cod liver oil	2
$Ca_2(PO_4)_2$	1
$CaCO_3$	1
NaCl	1
Thiamin	50 gamma per K per day
Riboflavin	" " " " " "

TABLE I

NICOTINIC ACID POTENCY OF FOODS BASED ON BIOASSAYS WITH DOGS

Material	*Mg. per gm. dry weight*	*Material*	*Mg. per gm. dry weight*
Liver, pork	1.2	Tongue, beef	0.4-0.5
Liver, lamb	1.2	Veal	0.5
Liver, veal	0.9	Brain, beef	0.3-0.5
Kidney, pork	0.85-1.0	Heart, pork	0.3
Yeast, brewers	1.0	Heart, beef	0.3
Yeast, bakers	0.5	Dried cereal grasses	0.1-0.15
Pork, loin	0.45-0.6	Skim milk powder	.05-.15
Pork, ham	0.4	Wheat germ	.05-.1

The response of each dog to standard amounts of nicotinic acid was determined before any assays were started and again after two or three assays had been made. The response measured was that obtained from a single dose of food material, all of which was consumed within a period of 24 hours.

Table I summarizes some of our results calculated as milligrams of nicotinic acid per gram of dry food. Our results are somewhat higher than those given by Bandier. It is entirely possible that the response shown by dogs when given liver as a source of nicotinic acid is somewhat greater than that resulting from nicotinic acid alone, but we must not overlook the possibility of incomplete extraction in the chemical procedures. If we assume the daily human requirement for nicotinic acid is 25 mg., then 25 gm. of dry liver or one-quarter of a pound of fresh liver will supply the requirement. About one-half pound of lean meat per day will amply meet the need.

In the assay work it became apparent immediately that only certain foods will produce rapid improvement when given at levels which will be consumed readily by the sick dog. This group includes practically all animal tissues and yeast. Foods like wheat germ and skim milk gave a response only when the supplements were continued over a period of several days. The availability of a reliable chemical method will greatly facilitate the work on the distribution of this vitamin in foods. In gen-

TABLE II

ANTI-BLACK TONGUE ACTIVITY OF VARIOUS PYRIDINE DERIVATIVES

Active	*Inactive*
Nicotinic acid	Pyridine
Nicotinic acid amide	Picolinic acid
Ethyl nicotinate	Isonicotinic acid
Nicotinic acid N methyl amide	Nipecotic acid
Nicotinic acid N diethyl amide	6-Methyl nicotinic acid
β-Picoline	Trigonelline
Nicotinuric acid	1-Methyl nicotinic acid amide chloride
	Quinolinic acid
	β-Aminopyridine

eral we may say that most natural foods will vary from 1 to 100 mg. of nicotinic acid per 100 gm. dry material. Certain crude liver extracts may contain as high as 200 to 300 milligrams per 100 grams. In order to supply amounts of nicotinic acid which have proved effective in the treatment of pellagra, it would be necessary to feed at least 100 grams of the more concentrated sources.

As long as severe pellagra is encountered, I imagine the use of nicotinic acid or related compounds will have to be continued, but when it is used we must remember that there are at least five other members of the B complex which may also be low in the diet of pellagrins. Work both in the field and with dogs on the Goldberger diet indicates a possible deficiency of thiamin and riboflavin.

Dogs grow very well on the Goldberger diet supplemented with thiamin, riboflavin and nicotinic acid, but if the corn in such a diet is replaced by sucrose the dogs will grow for only a short time and then begin to lose weight. The addition of 2 per cent of liver extract renders the diet complete. We are still working on the fractionation of liver extract, but to date we are certain that the liver extract must supply at least three additional factors, vitamin B_6, factor W, and pantothenic acid. If the dogs are deprived of vitamin B_6 they will develop a severe microcytic anemia which responds readily to the administration of 60 micrograms of synthetic vitamin B_6 per day. When the basal is supple-

mented with vitamin B_6 alone there is no growth until pantothenic acid and factor W concentrates are added.

Thus it is important to follow the nicotinic acid therapy with foods rich in other members of the B complex. Nicotinic acid as such is an emergency measure. Our goal should be the modification of the diet so that the people existing on marginal diets would obtain nicotinic acid as well as other essentials from foods. This does not mean that certain foods low in nicotinic acid but relatively rich in other essentials could not be fortified when experimental work has shown the proper means of fortification. The dogmatic statement that foods must not be fortified with nicotinic acid or other synthetic vitamins should not be accepted until all the facts are available.

Let us now turn to the second question: Are other compounds equally as effective as nicotinic? Shortly after we demonstrated the effectiveness of nicotinic acid in the dog, Mr. Madden and I, in conjunction with Strong and Woolley,[47] tested the activity of a number of related pyridine derivatives. The active and inactive compounds are listed in Table II. It was immediately apparent that a rather specific structure is required for anti-black tongue potency. The alpha and gamma isomers of nicotinic acid (picolinic acid and isonicotinic acid) are completely inactive. All the compounds tested in which one of the ring hydrogens had been substituted (by a methyl or a carboxyl group) or in which a methyl group had been added to the ring nitrogen were inactive. The replacement of the carboxyl group of nicotinic acid by a sulfonic acid group or by a cyano group or the removal of the carboxyl entirely (i.e., pyridine) led in each case to inactive compounds.

It appears probable that in addition to the acid and its amide only those compounds possess anti-black tongue potency which are capable of oxidation on hydrolytic conversion to these substances in the body. Evidence for this view is based on the fact that alkyl substituted amide proved to be active as did the ethyl ester. β-picoline which might be expected to be oxidized in the body to nicotinic acid showed a fair degree of activity. Nicotinuric acid was also active, which indicates that the body can hydrolyze this dipeptide. Subbarow, Dann, and Meilman[48] reported in a preliminary note that β-aminopyridine was highly active in the treatment of black tongue. When this compound was tried in our laboratory, we[49] found it to be completely inactive. In a later note Subbarow and Dann[50] confirmed our findings.

It is interesting that there is a close correlation between the results obtained in our work with dogs and those obtained by Dorfman, Koser, and Saunders[51] with the dysentery bacillus. Since they found β-picoline to be completely devoid of growth-promoting activity the organisms evidently cannot oxidize the methyl group while the animal body can bring about this transformation to a limited extent.

Recently there has been considerable interest in the activity of related pyrazine and thiazole compounds. Dr. Bills has prepared both the mono and the 2,3 dicarboxylic acid derivatives of pyrazine, and Spies (personal communication) has found that they show some activity in pellagra. We have also found that they possess about one-tenth the activity of nicotinic acid in the treatment of black tongue. Dr. Schmelkes has sent us thiazole 5 carboxylic and it also shows some biological activity. To date no compound has been found which is more effective than nicotinic acid or the amide.

At this point we should say a word about the toxicity of nicotinic acid. The work of both Chen, Rose and Robbins[52] and of Unna[53] indicates the very low toxicity of nicotinic acid and its derivatives. Sodium nicotinate showed a toxicity in mice and rats only when fed at levels ranging from 4 to 7 grams per kilogram body weight. The amide was found to be somewhat more toxic than the sodium salt. Unna found that prolonged oral administration of 2 grams per kilogram daily of sodium nicotinate to rats, chickens, and dogs over periods up to 2 months failed to produce toxic symptoms. The work of Chen, as well as that in our own laboratory showed that dogs receiving 2 gm. of nicotinic acid per day for several days showed some toxicity. However, Unna suggests that the toxicity may have been due to the acidity of the nicotinic acid since he observed no ill effects with the neutralized compound in even larger doses. In any case there seems to be the same wide range between therapeutic dose and toxic dose for nicotinic acid as for the other vitamins.

In addition to the above results practically all investigators have found that the administration of large amounts of nicotinic acid to human beings is often followed by sensations of heat and tingling of the skin. This feeling is accompanied by flushing and rise in skin temperature. In normal individuals the intravenous injection of 20 mg. nicotinic acid or its salt will cause an increased skin temperature. According to Spies, Bean, and Ashe[32] the chemicals effective in pellagra therapy do

not always produce flushing but those provoking the temperature rise are all effective therapeutic agents. Sebrell and Butler[54] found reactions in some persons on continued treatment with daily doses as low as 30 mg. by mouth. They conclude that the occurrence of these transient reactions should not be allowed to interfere with the therapeutic use of large doses of nicotinic acid.

As soon as the nutritional significance of nicotinic acid was recognized, it was generally assumed that its function in the animal body must be related to coenzymes I and II. However, it was not easy to obtain direct evidence for this relationship. Both coenzymes are very important in carbohydrate metabolism and they are supposed to differ in structure only by one molecule of phosphoric acid, yet they possess remarkable specificity in relation to the dehydrogenases with which they will react. In most cases a substrate together with its dehydrogenase will react with one of the coenzymes but not with the other. The quantitative estimation of the amount of coenzyme present in tissues is based upon this specificity. Of the two factors, coenzyme I or cozymase is most easily estimated.

The most obvious approach to any study on the function of nicotinic acid is therefore the estimation of the coenzyme content of the tissues from animals suffering from nicotinic acid deficiency. Euler and co-workers[55] used this approach on rats but unfortunately a specific nicotinic acid deficiency was not produced in the rats. Very recently Euler and co-workers[56] have presented results to show that tissues from rats on diets low in the B complex tend to be lower in nicotinamide and cozymase than those from normal rats. However, it remains difficult to demonstrate an uncomplicated deficiency of the pyridine nucleotides or any of their precursors in the rat. We have, therefore, used the dog and the pig in all of our studies.

We have limited our work to coenzyme I and the amount in various tissues has been determined by the yeast fermentation method which has been employed by the Euler group. The method is based on the principle that the addition of varying amounts of coenzyme to a washed yeast preparation will produce rates of fermentation which are proportional, within certain limits, to the amount of coenzyme I added. The rate of CO_2 evolution is measured in a Barcroft differential manometer under very carefully controlled conditions. The optimum levels of washed yeast, glucose, magnesium, manganese, hexose diphosphate, and

TABLE III

THE COENZYME I CONTENTS (EXPRESSED IN MICROGRAMS PER GM. OF FRESH WEIGHT) OF VARIOUS TISSUES

Animal	*Liver*	*Kidney cortex*	*Brain gray matter*	*Gastrocnemius muscle*	*Blood*
Guinea pig	523 (4)*	503 (4)	107 (4)	662 (4)	65-89 (4)
Rat	1114 (6)	1077 (6)	353 (6)	782 (6)	84-106 (6)
Chicken	878 (3)	990 (3)	306 (3)	693 (3)	65-105 (15)
Dog	1185 (1)	1060 (1)	—	458 (1)	51-66 (4)
Human	—	—	—	—	20-35 (10)

* Figures in parentheses indicate the number of animals used.

buffer must be determined for each type of yeast used. Under these conditions the cozymase content of animal tissues may be determined by comparison with standard amounts of pure cozymase provided there is no loss of cozymase during the preparation of the animal tissues for analysis.

Dr. Axelrod, working in our laboratory, has found that the following procedure allows practically complete recovery of the cozymase. The animal is sacrificed in the absence of anesthesia and the desired tissue removed immediately, cut into thin slices, and placed on slabs of solid carbon dioxide. The frozen tissue is ground to a fine powder, placed in boiling water, boiled for 2 minutes, and cooled immediately. A suitable aliquot can then be taken for analysis. When blood is analyzed the red cells are separated and placed directly in hot water.

In Table III are given the results obtained with several different tissues taken from guinea pigs, rats, chickens, dogs, and humans. It is apparent that cozymase content of the tissues from different species does not vary greatly in spite of the apparent difference in the nutritional requirements. The values given in this table are somewhat higher than those found in the literature due undoubtedly to the fact that great care was taken to prevent destruction of the cozymase during the process of extraction.

Our first studies on the effect of a nicotinic acid deficiency involved the determination of the cozymase content of the blood of normal dogs

TABLE IV

THE EFFECT OF A NICOTINIC ACID DEFICIENCY UPON THE COENZYME I CONTENT OF DOG TISSUES

Tissue	*Micrograms of coenzyme I per gram of fresh tissue*		
	Dog IV deficient	*Dog V deficient*	*Dog VI normal*
Kidney cortex	1130	1070	1000
Blood*	60	66	61
Liver	714	650	1185
Gastrocnemius muscle	295	427	490

* No significant changes were found in the hematocrit values.

and dogs suffering from black tongue. Throughout our entire work we have been unable to show any decrease in the blood even in very severe cases of black tongue. Similar results have been obtained with blood from pigs suffering from severe nicotinic acid deficiency. It is interesting that values for dogs range from 50 to 60 micrograms of cozymase per cc. of whole blood and for pigs the value is less than 10 micrograms. In both cases all of the cozymase was found in the corpuscles.

Vilter, Vilter, and Spies[45] using the method of Lwoff found a decrease in the cozymase content of the blood of pellagrins and an increase upon the administration of nicotinic acid. Kohn,[46] however, found no difference between the blood of normal and pellagrous patients but did find an increase even in normal patients upon administration of nicotinic acid. Our studies on humans carried out in coöperation with Dr. Edgar Gordon agree with those of Kohn. The value in normal patients was found to be 20-30 micrograms per cc. of blood, which may increase to 50-60 micrograms upon the ingestion of 100 mg. nicotinic acid per day. When the high level is established and the nicotinic acid is no longer supplied, the cozymase gradually decreases to the normal level during a period of about two weeks.

Our next approach was the estimation of cozymase in the tissues taken from the various animals. At first we did not have pure cozymase as a standard, but even in these studies it was evident that both the liver

TABLE V

COZYMASE AND NICOTINIC ACID CONTENT OF BIOLOGICAL MATERIALS

	Cozymase mg. per 100 gm. fresh wt.	*Nicotinic acid calculated from cozymase, mg. per 100 gm. fresh wt.*	*Nicotinic acid found, mg. per 100 gm. fresh wt.*
Liver, beef	70	12.9	26.1
Liver, pork	52	9.6	33.0
Muscle, beef	31	5.7	13.8
Muscle, rat	20*	3.7	——
Blood, human	2-3	.36-.54	.38**
Yeast, brewers	50*	9.2	24.0
Yeast, bakers	25*	4.6	12.0

* Euler — ** Swaminathan

and muscle tissue from deficient dogs contained considerably less than the same tissues from normal dogs. No differences could be detected in the kidney and brain. After the pure cozymase was made available to us by Dr. von Euler, it was possible to calculate the actual amount in the tissues.

The results obtained with one normal dog and two black tongue dogs are given in Table IV. From these figures it is evident that there may be a significant decrease in the liver and muscle depending upon the severity of the symptoms. Similar results have been obtained with pigs. The muscle of the deficient pigs showed a much greater decrease than in the case of the dogs.

It is significant that the brain, kidney, cortex, and blood maintained their normal coenzyme I content under the conditions of our experiments. Perhaps a normal level in these tissues is absolutely essential and a decrease is incompatible with life. In the case of liver and muscle, the vital functions can apparently be maintained although presumably to a greatly reduced degree in the absence of their normal content of coenzyme I. Under more severe conditions changes may also take place in tissues other than the liver and muscle. Whether the decrease in the liver and muscle is sufficient to account for the gross symptoms observed

cannot be answered at this time. However, it is quite probable that the rapid improvement noted both in humans and animals when nicotinic acid is administered is due to the rapid formation of cozymase when the nicotinic acid part of the molecule is made available.

Further evidence for the direct relationship of nicotinic acid found in animal tissues is present as cozymase. We have determined the cozymase content of a number of edible tissues which were also assayed for nicotinic acid potency on dogs. Some of these values are shown in Table V.

If the cozymase is converted to an equivalent amount of nicotinic acid we find that this amount makes up an appreciable amount of the total nicotinic acid. Part of the nicotinic acid must be present as coenzyme II and there is undoubtedly some free nicotinic acid and amide. More definite comparisons can be made when accurate chemical methods for these tissues are available.

In summary we may say that nicotinic acid has a history very similar to that of thiamin and riboflavin. The availability of pure nicotinic acid has not only given us a practical means of combating pellagra but has aided us in understanding some of the metabolic processes which are disturbed during pellagra. Such an understanding will aid in the diagnosis of a nicotinic acid deficiency before the gross symptoms of pellagra develop. We may look for similar results with vitamin B_6 and pantothenic acid as well as other unrecognized factors.

REFERENCES

1. Huber, C. Vorläufige Notiz über einige Derivate des Nicotins, *Liebig's Ann. Chem. u. Pharm.*, 1867, *141:* 271.
2. Funk, C. Studies on beri-beri; chemistry of the vitamine-fraction from yeast and rice-polishings, *J. Physiol.*, 1913, *46:* 173.
3. Suzuki, U., Shimamura, T. and Odake, S. Über Oryzanin, ein Bestandteil der Reiskleie und seine physiologische Bedeutung, *Biochem. Ztschr.*, 1912, *43:* 89.
4. Williams, R. R. The structure of the curative modifications of the hydroxypyridins, *J. Biol. Chem.*, 1917, *29:* 495.
5. Jahns, E. Ueber die Alkaloïde des Bockshornsamens, *Ber. d. deutsch. chem. Gesellsch.*, 1885, *18:* 2518.
6. Ackermann, D. Über das Vorkommen von Trigonellen und Nikotinursäure im Harn nach Verfütterung von Nikotinsäure, *Ztschr. f. Biol.*, 1912, *59:* 17.
7. Szymanska, R. M. and Funk, C. Die Wirkung von einigen Pyridinderivaten auf reisgefütterte Tauben, *Chem. d. Zelle u. Gewebe*, 1926, *13:* 44.
8. Warburg, O. and Christian, W. Co-Fermentproblem, *Biochem. Ztschr.*, 1934-35, *275:* 464.
9. Kuhn, R. and Vetter, E. Isolierung von Nicotinsäure-amid aus Herzmuskel, *Ber. d. deutsch. chem. Gesellsch.*, 1935, *68:* 2374.
10. von Euler, H., Albers, H. and Schlenk, F. Über die Co-Zymase, *Ztschr. f. physiol. Chem.*, 1935, *237:* 1.
11. von Euler, H. and Malmberg, M. Aktivatoren des Kohlenhydratabbaues als

wasserlösliche Nahrungskomponenten, *Biochem. Ztschr.*, 1936, *284:* 455.

12. Funk, C. and Funk, I. C. The value of pyridine derivatives in nutrition, *J. Biol. Chem.*, 1937, *119:* xxxv.
13. Frost, D. V. and Elvehjem, C. A. Further studies on factor W, *J. Biol. Chem.*, 1937, *121:* 255.
14. Sebrell, W. H. Table showing pellagra-preventive value of various foods, *Pub. Health Rep.*, 1934, *49:* 754.
15. Goldberger, J. and Sebrell, W. H. Blacktongue preventive value of Minot's liver extract, *Pub. Health Rep.*, 1930, *45:* 3064.
16. Spies, T. D. Pellagra: improvement while taking so-called "pellagra-producing" diet, *Am. J. M. Sc.*, 1932, *184:* 837.
17. Smith, D. T. and Ruffin, J. M. Treatment of pellagra with liver extracts, *J. Clin. Investigation*, 1933, *12:* 963.
18. Salmon, W. D. and Guerrant, N. B. Liver extract as a source of vitamins B and G_1, *Science*, 1931, *73:* 243.
19. Elvehjem, C. A. and Koehn, C. J., Jr. Studies on vitamin B_2 (G); non-identity of vitamin B_2 and flavins, *J. Biol. Chem.*, 1935, *108:* 709.
20. Kuhn, R., György, P. and Wagner-Jauregg, T. Eine neue Klasse von Naturfarbstoffen, *Ber. d. deutsch. chem. Gesellsch.*, 1933, *66:* 317; 576; 1034; 1577.
21. Lepkovsky, S. and Jukes, T. H. Vitamin G requirements of the chick, *J. Biol. Chem.*, 1935, *111:* 119.
22. Koehn, C. J., Jr. and Elvehjem, C. A. Studies on vitamin G (B_2) and its relation to canine black tongue, *J. Nutrition*, 1936, *11:* 67.
23. Birch, T. W., György, P. and Harris, L. J. Vitamin B_2 complex, *Biochem. J.*, 1935, *29:* 2830.
24. Dann, W. J. Vitamin G complex, *J. Nutrition*, 1936, *11:* 451.
25. Spies, T. D. *Personal communication.*
26. Fouts, P. J., Lepkovsky, S., Helmer, O. M. and Jukes, T. H. Successful treatment of human pellagra with "filtrate factor," *Proc. Soc. Exper. Biol. & Med.*, 1936-37, *35:* 245.
27. Elvehjem, C. A. *et al.* Isolation and identification of anti-black tongue factor, *J. Biol. Chem.*, 1938, *123:* 137.
28. Street, H. R. and Cowgill, G. B. Cure of canine blacktongue with nicotinic *acid, Proc. Soc. Exper. Biol. & Med.*, 1937-38, *37:* 547.
29. Dann, W. J. Nicotinic acid and vitamin B_2, *Science*, 1937, *86:* 616.
30. Sebrell, W. H. *et al.* Nicotinic acid in prevention of blacktongue in dogs, *J. Nutrition*, 1938, *16:* 355.
31. Mickelsen, O., Waisman, H. A. and Elvehjem, C. A. The inactivity of nicotinic acid in chick dermatitis, *J. Biol. Chem.*, 1938, *124:* 313.
32. Spies, T. D., Bean, W. B. and Ashe, W. F. Recent advances in the treatment of pellagra and associated deficiencies, *Ann. Int. Med.*, 1939, *12:* 1830.
33. Cleckley, H. M., Sydenstricker, V. P. and Geeslin, L. E. Nicotinic acid in the treatment of atypical psychotic states associated with malnutrition, *J.A.M.A.*, 1939, *112:* 2107.
34. Katzenellenbogen, I. Nicotinic acid in endemic glossitis, *Lancet*, 1939, *1:* 1260.
35. Landor, J. V. Deficiency of vitamin B_1, *Lancet*, 1939, *1:* 1368.
36. Sebrell, W. H. and Butler, R. E. Riboflavin deficiency in man, *Pub. Health Rep.*, 1938, *53:* 2282.
37. Karrer, P. and Keller, H. Eine kolorimetrische Bestimmung des Nicotinsäureamids, *Helv. chim. Acta*, 1938, *21:* 463.
38. Swaminathan, M. Chemical method for the estimation of nicotinic acid in biological materials, *Indian J. M. Research*, 1938, *26:* 427.
39. Shaw, G. E. and MacDonald, C. A. Colorimetric estimation of nicotinic acid as applied to commercial liver extracts, *Quart. J. Pharm. & Pharmacol.*, 1938, *9:* 380.
40. Bandier, E. and Hald, J. Colorimetric reaction for quantitative estimation of nicotinic acid, *Biochem. J.*, 1939, *33:* 264.
41. Bandier, E. Quantitative estimation of nicotinic acid in biological material, *Biochem. J.*, 1939, *33:* 1130.
42. Knight, B. C. J. G. Nutrition of Staphylococcus aureus; nicotinic acid and vitamin B_1, *Biochem. J.*, 1937, *31:* 731.
43. Mueller, J. H. Nicotinic acid as growth accessory for diphtheria bacillus, *J. Biol. Chem.*, 1937, *120:* 219.

44. Koser, S. A., Dorfman, A. and Saunders, F. Nicotinic acid as essential growth-substance for dysentery bacilli, *Proc. Soc. Exper. Biol. & Med.*, 1938, *38:* 311.
45. Vilter, R. W., Vilter, S. P. and Spies, T. D. Relationship between nicotinic acid and codehydrogenase (cozymase) in the blood of pellagrins and normal persons, *J.A.M.A.*, 1939, *112:* 420.
46. Kohn, H. I. Concentration of enzyme-like substance in the blood following administration of nicotinic acid to normal individuals and pellegrins, *Biochem. J.*, 1938, *32:* 2075.
47. Woolley, D. W., Strong, F. M., Madden, R. J. and Elvehjem, C. A. Anti-black tongue activity of various pyridine derivatives, *J. Biol. Chem.*, 1938, *124:* 715.
48. Subbarow, Y., Dann, V. J. and Meilman, E. The effect of β-aminopyridine in experimental blacktongue, *J. Am. Chem. Soc.*, 1938, *60:* 1510.
49. Strong, F. M., Madden, R. J. and Elvehjem, C. A. The ineffectiveness of β-aminopyridine in blacktongue, *J. Am. Chem. Soc.*, 1938, *60:* 2564.
50. Subbarow, Y. and Dann, W. J. The inactivity of β-aminopyridine in blacktongue, *J. Am. Chem. Soc.*, 1938, *60:* 2565.
51. Dorfman, A., Koser, S. A. and Saunders, F. The activity of certain nicotinic acid derivatives as growth essential for the dysentery bacillus, *J. Am. Chem. Soc.*, 1938, *60:* 2004.
52. Chen, K. K., Rose, C. L. and Robbins, E. B. Toxicity of nicotinic acid, *Proc. Soc. Exper. Biol. & Med.*, 1938, *38:* 241.
53. Unna, K. Studies on the toxicity and pharmacology of nicotinic acid, *J. Pharmacol. & Exper. Therap.*, 1939, *65:* 95.
54. Sebrell, W. H. and Butler, R. E. Reaction to oral administration of nicotinic acid, *J.A.M.A.*, 1938, *111:* 2286.
55. von Euler, H. *et al.* Zur Kenntnis der Cozymase-Bilanz im Rattenkörper, *Ark. f. Kemi., Mineral. o. Geol.*, 1938, *12A*, No. 25.
56. von Euler, H. *et al.* Nicotinsäureamid und Cozymase in normalen und in avitaminotischen Ratten, *Ztschr. physiol. Chem.*, 1939, *258:* 212.

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Oral Hygiene Committee of Greater New York. *Radio manual; a compilation of radio broadcasts for mouth health education.*
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Smith, L. W.; Weiss, E.; Lillie, W. I. [*et al.*]. *Cardiovascular-renal disease.*
N. Y., Appleton-Century, [1940], 227 p.

Smith, S. A. *Forensic medicine.* 6. ed.
Boston, Little, 1939, 654 p.

Trattato di radiobiologia diretto dal Prof. R. Balli.
Roma, "Universitas" Società Editrice, 1939, v. 1 & 2.

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Warren, L. H. *Handbook of skin diseases.*
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Wolter, F. *Vergleichende Epidemiologie.*
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PROCEEDINGS OF ACADEMY MEETINGS

STATED MEETINGS

JANUARY 4—*The New York Academy of Medicine. Annual Meeting.* Executive session—a] Reading of the Minutes; b] Presentation of diplomas. ¶ Presentation of annual reports (to be read by title) the Council, the Trustees, the Treasurer, Committees. ¶ Papers of the evening. Chemotherapy including sulfapyridine and allied compounds—a] General considerations, Francis G. Blake, Sterling Professor of Medicine, Yale University School of Medicine; b] Pneumonia, Norman Plummer, Instructor in Medicine, Cornell University School of Medicine; c] Toxic effects, William S. Tillett, Professor of Medicine, New York University College of Medicine. ¶ Report on election of members.

JANUARY 18—*The Harvey Society in affiliation with The New York Academy of Medicine.* The fourth Harvey lecture, "Heredity and the Development of Early Abnormalities in Vertebrates," L. C. Dunn, Professor of Zoology, Columbia University.

SECTION MEETINGS

JANUARY 2—*Dermatology and Syphilology.* Presentation of cases—a] New York University, College of Medicine and Bellevue Hospital; b] Miscellaneous cases. ¶General discussion. ¶ Executive session.

JANUARY 5—*Surgery.* Reading of the minutes. ¶Presentation of cases—a] Recurrent acute pancreatitis, James Edwin Thompson; b] Recurrent acute pancreatitis, Frederick Henry Amendola; Discussion, Henry Wisdom Cave; c] Primary adenocarcinoma of the jejunum with resection, Nelson Warren Cornell; Discussion, N. Chandler Foot; d] Cyst of the pancreas, complete excision; Result after 6 months, John H. Garlock; Discussion, Frank J. McGowan. ¶ Papers of the evening—a] Clinical value of serum amylase studies in pancreatic disease, Edward F. Lewison (by invitation); Discussion, Percy Klingenstein; b] Carcinoma of the head of the pancreas, William Barclay Parsons; Discussion, William Crawford White; c] Studies in pancreaticogastrostomy and partial pancreatectomy in animals, Frank Glenn; Discussion, William Frank MacFee. ¶ General discussion. ¶ Executive session.

JANUARY 9—*Combined Meeting, Section of Neurology and Psychiatry and the New York Neurological Society.* Papers of the evening—a] Cerebral arteriography by means of a rapidly excreted organic iodide compound, Sidney W. Gross; Discussion, Cornelius G. Dyke, Irvine H. Marshall (by invitation), Lawrence Poole (by invitation); b] Recent experimental disclosures concerning the functions of the frontal lobes, John F. Fulton (by invitation); Discussion, Richard M. Brickner, Lauretta Bender, Kurt Goldstein (by invitation), John E. Searff; c] Pupillography; its significance in clinical neurology, Otto Loewenstein (by invitation); Discussion, John F. Fulton (by invitation), M. Davidson, Bernard Sachs. ¶ Executive session.

JANUARY 10—*Historical and Cultural Medicine.* Reading of the minutes. ¶ Paper of the evening—The Renaissance of anatomy, from Mondino to Leonardo, Professor A. Castiglioni, Padua, Italy (by invitation). ¶ Discussion, Victor Robinson (by invitation), Leona Baumgartner (by invitation), George Rosen (by invitation). ¶ General discussion.

JANUARY 11—*Pediatrics.* Program presented by members of the Department of Pediatrics of Harvard University Medical School. Reading of the minutes. ¶ Papers of the evening—a] Blood ascorbic acid concentrations in terms of plasma red

cells and white cells, Allan M. Butler (by invitation); b] Extracellular fluid volume, James L. Gamble (by invitation); c] Variability of the clinical and hematological manifestations of leukemia in childhood, Louis K. Diamond (by invitation); d] The criteria for ligation of the patent ductus arteriosus in the light of the first one and a half-years' experience, John P. Hubbard (by invitation), Robert E. Gross (by invitation). ¶ General discussion. ¶ Executive session.

JANUARY 15—*Ophthalmology.* Instruction hour 7:00 to 8:00—Non-suppurative diseases of the cornea, Ralph I. Lloyd. ¶ Demonstration of Slit Lamp Cases 8:00 to 8:30, Gordon M. Bruce, Girolamo Bonaccolto, Milton Berliner. ¶ Reading of the minutes. ¶ Presentation of cases—a] Motion picture demonstration of unusual neuro-ophthalmological conditions, S. Philip Goodhart, Benjamin Balser (by invitation); b] Repair of entropion, Raymond Meek. ¶ Papers of the evening—a] Diagnostic significance of central scotoma, Frank B. Walsh, Baltimore (by invitation); Discussion, John Evans; b] Sclerocorneal sutures, John M. McLean, Baltimore (by invitation). ¶ Executive session.

JANUARY 16—*Medicine.* Reading of the minutes. ¶ Papers of the evening—a] Trauma and its relationship to heart disease, Louis Faugeres Bishop; Discussion, Arthur M. Master; b] Weil's disease in New York City, Elliston Farrell (by invitation); Discussion. ¶ General discussion.

JANUARY 17—*Genito-Urinary Surgery.* Reading of the minutes. ¶ Papers of the evening. Symposium on Experimental and clinical phases of sex hormone therapy —a] Current errors about sex and sex hormones, James F. McCahey, Department of Urology, Jefferson Medical School (by invitation); b] Indications for hormone therapy in glandular deficiencies, Walter M. Kearns, Department of Urology, Marquette University (by invitation); c] Evaluation of hormone therapy in diseases of the genito-urinary system, Norris J. Heckel, Department of Urology, Rush Medical School (by invitation). ¶ General discussion, Earl T. Engle, Columbia University. ¶ Executive session.

JANUARY 17—*Otolaryngology.* Reading of the minutes. ¶ Papers of the evening—a] Dental reactions of the temporomandibular joint of interest to otolaryngologists (motion pictures, lantern slides), Sidney E. Riesner, D.D.S. (by invitation); Discussion, Edmund Prince Fowler, Sr., Solomon Fineman; b] Voice anomalies of hysteric origin. Psychosomatic interrelationship (slides, recordings), James Sonnett Greene; Discussion, Oskar Diethelm, Cornell University Medical College (by invitation), Irving W. Voorhees (by invitation). ¶ Executive session.

JANUARY 23—*Obstetrics and Gynecology.* Reading of the minutes. ¶ Papers of the evening—a] Potency evaluations of the commercial female hormone preparations, Bernard L. Cinberg (by invitation), Stanley F. Goldman (by invitation); Discussion, Edwin G. Langrock; b] Studies in artificial ovulation with the hormone of pregnant mares' serum (motion pictures in color), Samuel L. Siegler (by invitation); Discussion, Raphael Kurzrok.

Orthopedic Surgery—There was no meeting of the Section of Orthopedic Surgery in January because of the annual meeting of the American Academy of Orthopedic Surgeons which was held January 21-25, at the Hotel Statler, Boston.

AFFILIATED SOCIETIES

JANUARY 15—*New York Roentgen Society in affiliation with The New York Academy of Medicine.* Case reports—a] Skeletal changes induced by the administration of estrogen, Charles J. Sutro (by invitation); b] An unclassified destructive bone lesion simulating a malig-

nant tumor, Louis Lichtenstein (by invitation); c] A review of 58 cases of tabetic arthropathy, Maurice M. Pomeranz, Abraham S. Rothberg (by invitation). ¶ Paper of the evening—Findings in cases generally misinterpreted as cortical bone abscess or sclerosing osteomyelitis of long bones, Henry L. Jaffe. ¶ Discussion. ¶ Executive session.

JANUARY 25—*New York Pathological Society in affiliation with The New York Academy of Medicine.* Case reports—a] A case of thromboarteritis of the pulmonary artery with chronic obstruction in the pulmonary circulation, B. M. Vance, Chief Medical Examiner's Office; b] Contralateral adrenal atrophy associated with cortical adrenal neoplasm, Tobias Weinberg, Mt. Sinai Hospital. ¶ Papers of the evening—a] The influence of sulfanilamide and sulfapyridine upon the evolution of experimentally induced pneumococcus pneumonia in rats, David Goldstein (by invitation), Irving Graef, New York University College of Medicine; b] Aplastic anemia, Cornelius P. Rhoads (by invitation), Memorial Hospital. ¶ Executive session, election of officers.

DEATHS OF FELLOWS

BREWER, GEORGE EMERSON: 150 East 73 Street, New York City; born in Westfield, New York, July 28, 1861; died in New York City, December 24, 1939; received the degrees from Hamilton College of A.B., 1881; M.A., 1884; LL.D., 1916; M.D. from Harvard University in 1885; and hon. D.Sc. from Columbia University in 1929; elected a Fellow of the Academy October 3, 1889.

Dr. Brewer was emeritus professor of surgery at the College of Physicians and Surgeons from 1917 until the date of his death, having served that institution as assistant demonstrator in anatomy, 1892-1900; instructor in surgery 1900-1903; professor of clinical surgery, 1903-1914; and professor of surgery, 1914-1917. He was consulting surgeon to the Roosevelt, City, Woman's and Presbyterian Hospitals of New York City; Christ Hospital at Jersey City, Perth Amboy Hospital at Perth Amboy and Muhlenberg Hospital at Plainfield, New Jersey.

Dr. Brewer was a Fellow of the American College of Surgeons, and the Royal College of Surgeons (Ireland), a member of the American Surgical Association and its president in 1919, the New York Surgical Society, the American Society of Genito-Urinary Surgeons, the Society of Clinical Surgery and its president, 1901-04, Société Internationale de Chirurgie, Société Internationale d'Urologie, the American Medical Association and the New York County and State Medical Societies.

In 1917, Dr. Brewer went to France to direct Base Hospital No. 2 which relieved General Hospital No. 1 of the British Expeditionary Force at Etretat. He was promoted to the rank of chief surgeon of the 42nd Division and consultant in surgery to the First Corps, First Army of the American Expeditionary Force and later promoted to the rank of colonel.

Dr. Brewer was the author of a "Text Book on Surgery," and "Surgical Diagnosis," and many articles on anatomical and surgical conditions.

LEWIS, ROBERT: 40 East 64 Street, New York City; born in New York City, March 8, 1862; died in New York City, December 20, 1939; graduated in medicine from the College of Physicians and Surgeons in 1885;

elected a Fellow of the Academy May 6, 1897, served as Chairman of the Section of Otology in 1910, and designated a Fellow in Otolaryngology in 1933.

Dr. Lewis was clinical professor emeritus of otolaryngology at the College of Physicians and Surgeons, Columbia University, from July 1939 until his death, having served that institution as instructor in otology, 1896-1908; professor of clinical otology, 1908-1916; professor of clinical laryngology and otology, 1916-1929; professor of clinical otolaryngology, 1929-1938; and clinical professor of otolaryngology, 1938-1939. From 1901 until 1928, he was surgeon to the aural department of the New York Eye and Ear Infirmary, and in 1928 he was elected consulting surgeon. He was also consulting otolaryngologist to the New York Society for Relief of the Ruptured and Crippled, Flushing, St. Francis and Sea View Hospitals.

Dr. Lewis was a diplomate of the American Board of Otolaryngology; a Fellow of the American College of Surgeons and a member of its Board of Governors, 1921-1925; a member of the New York Otological Society and its president, 1911-1913; a member of the American Otological Society and its president in 1920; a member of the American Rhinological, Laryngological and Otological Society and its vice-president in 1919; a member of the American Medical Association and the New York County and State Medical Societies; and an alumnus of the Federation of Columbia University and its vice-president in 1919.

Dr. Lewis contributed treatises on otological topics to "Reference Handbook of Medical Sciences," to "The American Practice of Surgery" and to medical journals.

MENDELSON, WALTER: 639 Church Lane, Germantown, Philadelphia, Pennsylvania; born in Germantown, April 9, 1857; died in Germantown, January 18, 1940; graduated in medicine from the College of Physicians and Surgeons, Columbia University, in 1879; elected a Fellow of the Academy June 5, 1884.

Dr. Mendelson was a member of the Board of Trustees of Columbia University, 1913-1918, and served as Chairman of the Board of Trustees of the College of Physicians and Surgeons in 1923. He was president of the Alumni Association of the College of Physicians and Surgeons, 1910-1919, and was awarded the Alumni Medal of Columbia University, in 1933.